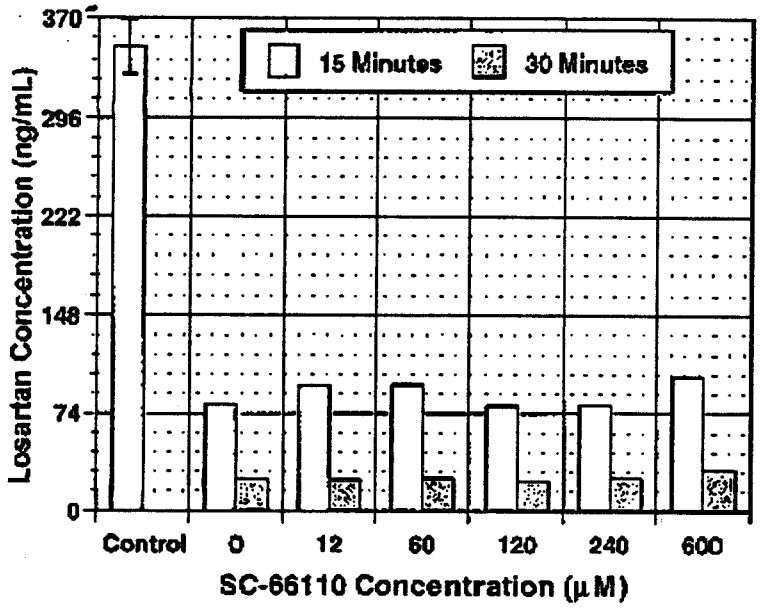
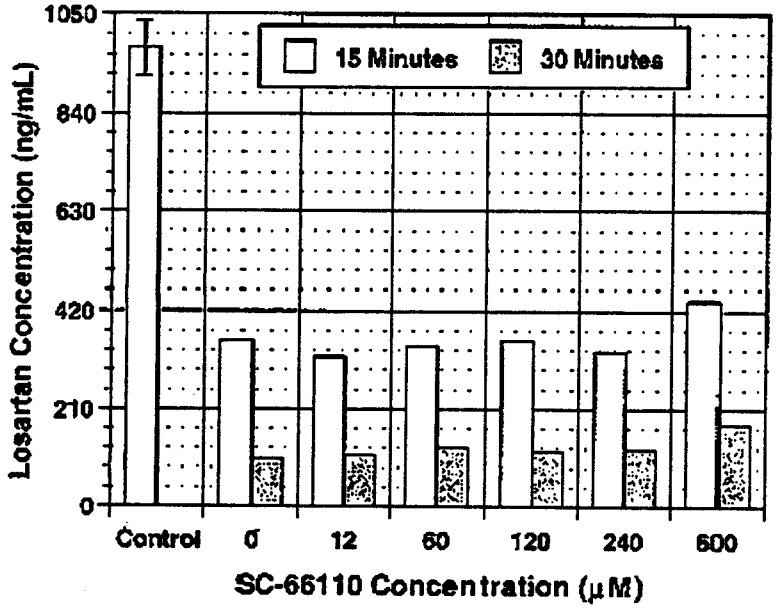
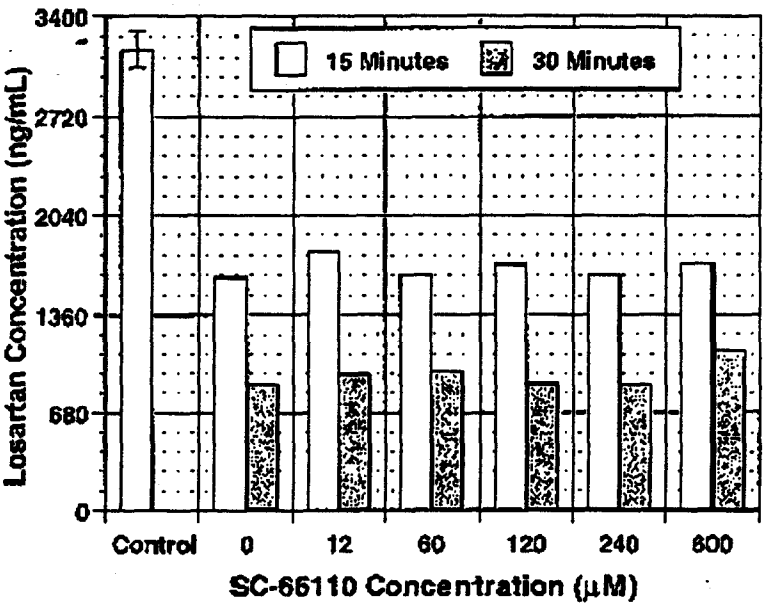


Effect of Eplerenone on the Depletion of Losartan (3.27 mM) During Incubation with Human Liver Microsomes



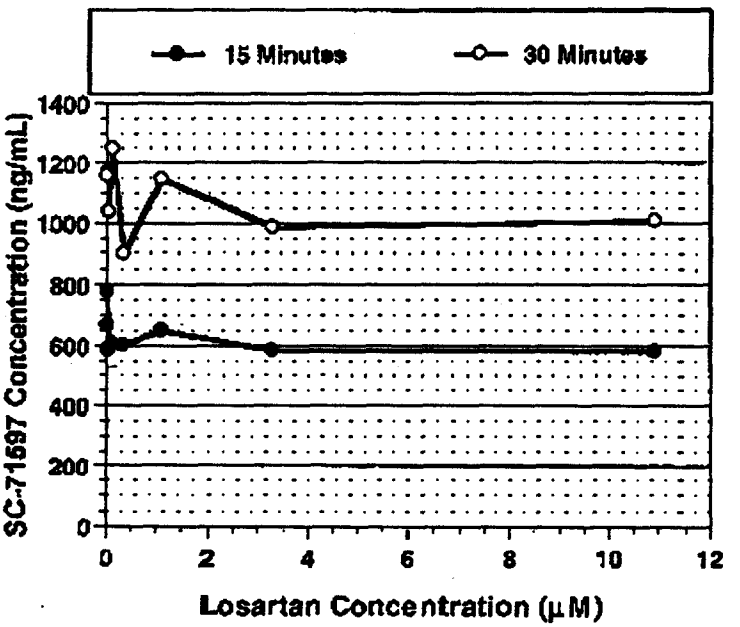
Effect of Eplerenone on the Depletion of Losartan (10.9 mM) During Incubation with Human Liver Microsomes



Effect of Losartan on Eplerenone Metabolism in Human Liver Microsomes

Formation of SC-71597 was not appreciably decreased (less than 20%) following 15 min or 30 minutes incubations in the presence of up to 10.9 µM losartan.

Effect of Losartan on the Formation of SC-71597 During Incubation of Eplerenone (12 mM) with Human Liver Microsomes



CONCLUSIONS

Eplerenone at concentrations up to 600 μM did not have a significant effect on the metabolism of losartan (measured by disappearance of parent compound), or on the formation of losartan carboxylic acid metabolite, in human liver microsomal suspensions.

Losartan concentrations up to 10.9 μM did not have a significant effect on the formation of SC-71597 metabolite of eplerenone in human liver microsomal suspensions.

INHIBITION OF CYTOCHROME P4501A2, CYTOCHROME P4502C9, CYTOCHROME P4502D6, CYTOCHROME P4502C19 AND CYTOCHROME P4503A4 CATALYTIC ACTIVITIES BY EPLERENONE

Document #: M2098121

OBJECTIVES:

To determine whether eplerenone inhibited selected human cytochrome P450 catalytic activities of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4.

METHODS:

Microsomes were obtained from metabolically competent human B-lymphoblastoid cell lines that express human cytochrome P450 for 1A2, 3A4, 2C9, and 2D6 or from baculovirus-insect cell-expressed CYP2C19. Control microsomes used to standardize assay protein concentrations, were obtained from a human B-lymphoblastoid cell line that contained no detectable cDNA-expressed cytochrome P450 catalytic activity. Each enzyme assay included eplerenone concentrations of 300, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 µM. Inhibitors were used as positive controls for inhibition of each enzyme as described below.

CYP1A2 catalytic activity was measured in assays containing 0.4 mg/ml CYP1A2 enzyme protein and 50 mM phenacetin in 100 mM potassium phosphate, pH 7.4. The assays were incubated at 37 o C for 30 min and stopped by the addition of 50 µl acetonitrile. The positive control for CYP1A2 was 7,8-benzoflavone run at final concentrations of 0.3 and 3 µM.

CYP2C9 catalytic activity was measured in assays containing 0.02 mg/ml CYP2C9 enzyme protein, 0.38 mg/ml control microsome protein, and 6 µM diclofenac in 100 mM Tris, pH 7.5. The assays were incubated at 37^{0}C for 30 minutes and stopped by the addition of 50 µl 94% acetonitrile-6% acetic acid. The positive control for CYP2C9 was sulfaphenazole run at final
concentrations of 0.3 and 3 µM.

CYP2C19 catalytic activity was measured in assays containing 0.08 mg/ml CYP2C19 enzyme protein, 0.32 mg/ml control microsome protein, and 50 µM [^{14}C]-(S)-mephenytoin (sp.act. = 5.16 mCi/mmole) in 50 mM potassium phosphate, pH 7.4. The assays were incubated at 37^{0}C for 20 minutes and stopped by the addition of 50 µl acetonitrile. The positive control for CYP2C19 was tranylcypromine run at final concentrations of 10 and 100 µM.

CYP2D6 catalytic activity was measured in assays containing 0.1 mg/ml CYP2D6 enzyme protein, 0.3 mg/ml control microsome protein, and 10 µM (±)-bufuralol in 100 mM potassium phosphate, pH 7.4. The assays were incubated at 37^{0}C for 30 minutes and

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stopped with 25 µl 70% perchloric acid. The positive control for CYP2D6 was quinidine run at final concentrations of 0.1 and 1 µM.

CYP3A4 catalytic activity was measured in assays containing 0.2 mg/ml CYP3A4 enzyme protein, 0.2 mg/ml control microsome protein, and 120 µM testosterone in 100 mM potassium phosphate, pH 7.4. The assays were incubated at 37°C for 30 minutes and stopped with 125 ml acetonitrile. Protein was removed by centrifugation. The positive control for CYP3A4 was ketoconazole run at final concentrations of 0.1 and 1 µM.

RESULTS

Inhibition of CYP1A2 Activity by Eplerenone

Eplerenone inhibited CYP1A2 catalytic activity by approx. 20% at 300 µM. No IC50 value was calculated because all inhibition values were below 50%. The positive control inhibitor, 7,8-benzoflavone, completely inhibited CYP1A2 activity even at the lowest concentration tested (0.3 µM).

Inhibition of CYP1A2 Catalytic Activity by Eplerenone

Concentration (µM)	Pmole per Incubation	Percent Inhibition
0	631, 620	-
0.01	582, 604	7, 4
0.03	583, 582	7, 7
0.1	609, 614	3, 2
0.3	603, 589	4, 6
1	624, 597	0, 5
3	616, 589	2, 6
10	590, 582	6, 7
30	556, 557	11, 11
100	548, 560	12, 10
300	471, 539	25, 14

Inhibition of CYP1A2 Catalytic Activity by the Positive Control Inhibitor, 7,8-Benzoflavone

Concentration (µM)	Pmole per Incubation	Percent Inhibition
0	631, 620	-
0.3	0, 0	100, 100
3.0	0, 0	100, 100

Inhibition of CYP2C9 Activity by Eplerenone

No inhibition of CYP2C9 catalytic activity by eplerenone was observed at concentrations up to 300 µM with most inhibition values being below 10%. The positive control inhibitor, sulfaphenazole, inhibited CYP2C9 activity by approx. 45% at 0.3 µM and about 90% at 3.0 µM.

Inhibition of CYP2C9 Catalytic Activity by Eplerenone

Concentration (μM)	Pmole per Incubation	Percent Inhibition
0	654, 630	-
0.01	608, 570	5, 11
0.03	613, 574	4, 11
0.1	675, 621	-5, 3
0.3	568, 576	12, 10
1	586, 633	9, 1
3	611, 618	5, 4
10	630, 674	2, -5
30	639, 678	0, -6
100	612, 648	5, -1
300	629, 592	2, 8

Inhibition of CYP2C9 Catalytic Activity by the Positive Control Inhibitor, Sulfaphenazole

Concentration (μM)	Pmole per Incubation	Percent Inhibition
0	654, 630	-
0.3	347, 364	46, 43
3.0	84, 60	87, 91

Inhibition of CYP2C19 Activity by Eplerenone:

Eplerenone did not inhibit CYP2C19 catalytic activity at concentrations up to 300 μM with all inhibition values being below 10%. The positive control inhibitor, tranylcypromine, inhibited CYP2C19 activity by 70% at 10 μM and >95% at 100 μM.

Inhibition of CYP2C19 Catalytic Activity by Eplerenone

Concentration (μM)	Pmole per Incubation	Percent Inhibition
0	2299, 2690	-
0.01	2429, 2402	3, 4
0.03	2447, 2334	2, 6
0.1	2441, 2337	2, 6
0.3	2285, 2445	8, 2
1	2500, 2310	0, 7
3	2495, 2387	0, 4
10	2459, 2353	1, 6
30	2505, 2409	0, 3
100	2465, 2452	1, 2
300	2295, 2318	8, 7

Inhibition of CYP2C19 Catalytic Activity by the Positive Control Inhibitor, Tranylcypromine

Concentration (μM)	Pmole per Incubation	Percent Inhibition
0	2299, 2690	-
10.0	721, 750	71, 70
100.0	55, 71	98, 97

Inhibition of CYP2D6 Activity by Eplerenone:

No inhibition of CYP2D6 catalytic activity by eplerenone was observed at concentrations up to 300 µM with most inhibition values being below 12%. Quinidine, the positive control inhibitor, inhibited CYP2D6 activity by nearly 90% at 0.1 µM, the lowest concentration tested.

Inhibition of CYP2D6 Catalytic Activity by Eplerenone

Concentration (µM)	Pmole per Incubation	Percent Inhibition
0	251, 242	-
0.01	238, 236	3, 4
0.03	236, 239	4, 3
0.1	233, 232	5, 6
0.3	226, 231	8, 6
1	237, 234	4, 5
3	230, 234	6, 5
10	225, 227	8, 8
30	233, 240	5, 2
100	221, 213	10, 13
300	216, 219	12, 11

Inhibition of CYP2D6 Catalytic Activity by the Positive Control Inhibitor, Quinidine

Concentration (µM)	Pmole per Incubation	Percent Inhibition
0	251, 242	-
0.1	28, 30	89, 88
1.0	5, 5	98, 98

Inhibition of CYP3A4 Activity by Eplerenone:

Eplerenone inhibited CYP3A4 catalytic activity by approx. 32% at 0.1 µM and approx. 45% at 300 µM. No IC50 value was calculated because all inhibition values were below 50%. CYP3A4 activity was inhibited more than 75% by 0.1 µM ketoconazole, the positive control inhibitor. Nearly 95% inhibition was demonstrated at 1.0 µM ketoconazole.

Inhibition of CYP3A4 Catalytic Activity by Eplerenone

Concentration (µM)	Pmole per Incubation	Percent Inhibition
0	846, 724	-
0.01	831, 751	-6, 4
0.03	797, 718	-2, 8
0.1	530, 624	32, 20
0.3	522, 609	34, 22
1	473, 510	40, 35
3	594, 640	24, 18
10	585, 543	26, 31
30	550, 663	30, 16
100	484, 553	38, 30

300	424, 445	46, 43

Inhibition of CYP3A4 Catalytic Activity by the Positive Control Inhibitor, Ketoconazole

Concentration (μM)	Pmole per Incubation	Percent Inhibition
0	846, 724	-
0.1	183, 188	77, 76
1.0	52, 45	93, 94

IC50 Values for the Inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 Catalytic Activities by Eplerenone

Enzyme	IC50 (μM)	IC50 (μg/mL)
CYP1A2	>300	> 124 μg/mL
CYP2C9	>300	> 124 μg/mL
CYP2C19	>300	> 124 μg/mL
CYP2D6	>300	> 124 μg/mL
CYP3A4	>300	> 124 μg/mL

CONCLUSIONS

Eplerenone did not inhibit (IC50 =>300 μM) CYP1A2, CYP2C9, CYP2C19 and CYP2D6. Eplerenone inhibited CYP3A4 catalytic activity by approx. 32% at 0.1 μM and approx. 45% at 300 μM.

COMMENTS:

Moderate inhibition of CYP3A4 is expected at therapeutic doses of eplerenone (100mg QD) since mean Cmax of eplerenone following a 100 mg dose is about 1.5 μg/mL, equivalent to 3.6 μM.

THE POTENTIAL OF SC-66110 (EPLERENONE) TO INDUCE CYTOCHROME P450 ENZYMES IN FRESHLY ISOLATED HUMAN HEPATOCYTES

Document #: M2099317

OBJECTIVES:

To investigate the potential for induction of cytochrome P450 3A4 (CYP3A4) by eplerenone assessed by the formation of 6β-hydroxytestosterone in isolated human hepatocytes.

METHODS:

Isolation and Preparation of Hepatocytes

Hepatocytes were isolated from a human liver of transplant quality which was processed within 24 hours of cross clamp time using standard perfusion and digestion procedures. Viability of the isolated hepatocytes was 89% as determined by trypan blue exclusion. The hepatocytes (0.850 x 10^6 live cells per well) were cultured in 12-well collagen-coated tissue culture plates in a total volume of 1.00 mL [redacted] induction plating medium including 5% fetal bovine serum (FBS). After 48 hours incubation, the hepatocytes had arranged into cords similar to hepatic acinar architecture. Phase I and phase II metabolic integrity of the hepatocytes was verified by dextromethorphan O-demethylation and 7-ethoxycoumarin O-deethylation or by 7-hydroxycoumarin glucuronidation, respectively.

Treatment of Hepatocytes with Potential Inducing Agents

Media (2 mL) containing enzyme inducing agents eplerenone, phenobarbital (100 μM), or dexamethasone (100 μM) were added to wells of hepatocyte cultures prepared as described above. Eplerenone concentrations tested were 0.100, 1.00, 10.0, or 100 μM. A sufficient number of wells were prepared so that quadriplicate wells of each treatment could be evaluated following 24, 48 or 72 hours exposure. Additional wells for each treatment/time period were prepared to evaluate the effect of the inducing agent on cell viability assessed by trypan blue exclusion. Phase I and phase II metabolic integrity of solvent treated control cells was verified at the end of the experiment by dextromethorphan O-demethylation and 7-ethoxycoumarin O-deethylation or by 7-hydroxycoumarin glucuronidation, respectively.

Measurement of Enzymatic Activity

The CYP3A4 enzymatic activity of the treated hepatocytes was determined using the substrate testosterone. At the end of each specified period of exposure to the potential inducing agents, the media were aspirated and replaced with 1.00 mL warmed medium containing the substrate testosterone (200 μM). After an additional 30 min incubation, 975 μL of the supernatant was collected and added to 2 mL ethyl acetate. Samples prepared after the 24, 48, and 72 hour exposure periods were thawed simultaneously, reconstituted with [redacted] μL mobile phase and analyzed by [redacted] The concentration

of 6β-hydroxytestosterone in each sample was quantitated using a weighted linear least squares regression line generated from spiked calibration standards.

RESULTS:

Effect of Treatment with Potential Inducing Agents on Enzymatic Activity

Treatment with all 3 inducing agents resulted in a time-dependent increase in CYP3A4 activity as measured by the formation of 6β-hydroxytestosterone. In all cases, little or no change in activity was noted in hepatocytes exposed to inducing agents for 24 hours. However, differences between treatments reached a level of significance at the 48 hour time point (ANOVA). Treatment with eplerenone, phenobarbital, and dexamethasone (100 μM) resulted in enzymatic activity of hepatocytes that was significantly increased after 48 hours exposure. Additional increases were noted after 72 hours exposure time. However, eplerenone concentrations below 10 μM did not induce CYP3A4 activity. Treatment of hepatocytes with dexamethasone resulted in the greatest amount of induction in enzyme activity over the 72 hour period.

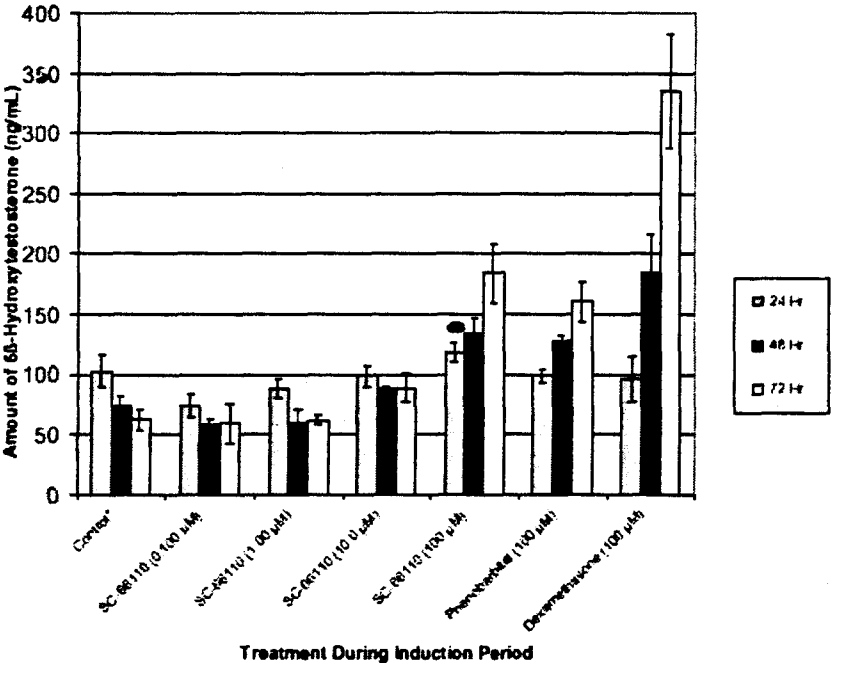
Effect of Potential Inducing Agents on CYP3A4 Activity in Primary Cultured Human Hepatocytes

Inducing Drug and Concentration	Time of Incubation with Potential Inducer +		
	24 Hr	48 Hr	72 Hr
Control*	103 + 13.7	74.8 + 6.49	62.7 + 9.04
Eplerenone (0.1 μM)	74.0 + 9.80	56.9 + 6.51	59.1 + 16.9
Eplerenone (1 μM)	87.9 + 7.33	60.5 + 9.57	61.5 + 4.58
Eplerenone (10 μM)	98.3 + 9.14	88.9 + 1.28	89.0 + 12.1
Eplerenone (100 μM)	118 + 7.86	134 + 13.3	184 + 24.3
Phenobarbital (100 μM)	98.3 + 5.73	127 + 5.57	160 + 17.5
Dexamethasone (100 μM)	96.4 + 19.0	185 + 30.5	335 + 47.1

+ Concentrations are the mean of four replicates (+ the Standard Deviation), reported as ng/ml of 6ß-Hydroxytestosterone formed during a 30 minute incubation conducted at the end of the induction period.
Control = 0.00 μM eplerenone.

Hepatocytes treated 72 hours with eplerenone or phenobarbital had activities approximately 3 times those of control cells. The activity in cells treated with dexamethasone reached nearly five times the activity in control treated cells and nearly 2 times the activity of cells treated with phenobarbital or with eplerenone (100 μM). These data indicate a potential for induction of CYP3A4 by eplerenone. In this in vitro study, the extent of induction in hepatocytes treated with eplerenone was approximately equivalent to induction by equimolar concentrations of phenobarbital but significantly less than induction due to similar concentrations of dexamethasone. It should be noted that both eplerenone and dexamethasone are substrates for CYP3A4 and that phenobarbital is a substrate for CYP2C9/19. The concentrations of the potential inducing agents remaining at the end of each incubation period were not monitored.

Effect of Potential Inducing Agents on CYP3A4 Activity in Primary Cultured Human Hepatocytes



CONCLUSIONS

Eplerenone concentrations below 10 μM did not induce CYP3A4 activity. However, eplerenone concentration of 100 μM significantly induced hepatocyte CYP3A4 activity, 3-fold increase compared to control, after 72 hours. The extent of induction in hepatocytes treated with eplerenone was approximately equivalent to induction by equimolar concentrations of phenobarbital but significantly less than induction due to similar concentrations of dexamethasone.

COMMENTS:

1. Eplerenone is not expected induce CYP 3A4 activity in vivo since the proposed therapeutic dose of 100 mg QD eplerenone results in eplerenone Cmax of about 1.5 μg/ml, equivalent to 3.6 μM, a concentration at which induction was not seen in vitro.
2. It is not clear whether the induction is caused by eplerenone or one of its metabolites. Induction is seen only at the highest eplerenone concentration of 100 μM, a concentration which yields a substantial amount of metabolite, indicating that a metabolite of eplerenone causes CYP3A4 induction.
3. The concentrations of the potential inducing agents remaining at the end of each incubation period were not monitored.

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND AMIODARONE

Document #: M2000327

OBJECTIVES:

To evaluate the potential for metabolic drug-drug interactions between eplerenone and amiodarone.

METHODS:

The metabolism of amiodarone was investigated in human liver microsomes, 0.25 mg/mL final concentration. A volume of 25 µL microsomes was added to 450 µL 100 mM potassium phosphate buffer pH 7.4. Eplerenone was added in a volume of 2 µL to final concentrations of 0, 1.00, 5.00, 25.0, 50.0 and 100 µM as appropriate and amiodarone was added in a volume of 4.00 µL to reach final concentrations of 200 or 1000 ng/mL. The enzymatic reactions were initiated by adding NADPH to a final concentration of 1 mM and the samples were incubated at 37 °C for 45 minutes. The reactions were quenched by the addition of mL of mobile phase.

Effect of Amiodarone on the Formation of SC-71597

A Ki quantifying inhibition of SC-71597 formation was estimated by incubating 5 eplerenone (substrate) concentrations with 6 concentrations of amiodarone (including zero). Human liver microsomes (25 µL) were added to 450 µL of 100 mM potassium phosphate buffer pH 7.4 to achieve a final protein concentration of 0.1 mg/mL. Eplerenone (2 µL in acetonitrile) was added to the appropriate suspensions to achieve the target concentrations of 25.0, 50.0, 100, 200 and 400 µM. Amiodarone was added to appropriate tubes and the suspensions were allowed to equilibrate for approximately 3 minutes. The concentrations used for amiodarone 0, 5, 12.5, 25.0, 50, and 125 µM . The enzymatic reactions were initiated by the addition of NADPH (25 µL) so that the final concentration was 1.00 mM. Incubations were quenched after 15 minutes by the addition of the extraction solvent ethyl acetate. The samples were injected onto the
The *m/z* 431→211 product ions of SC-71597 were monitored.

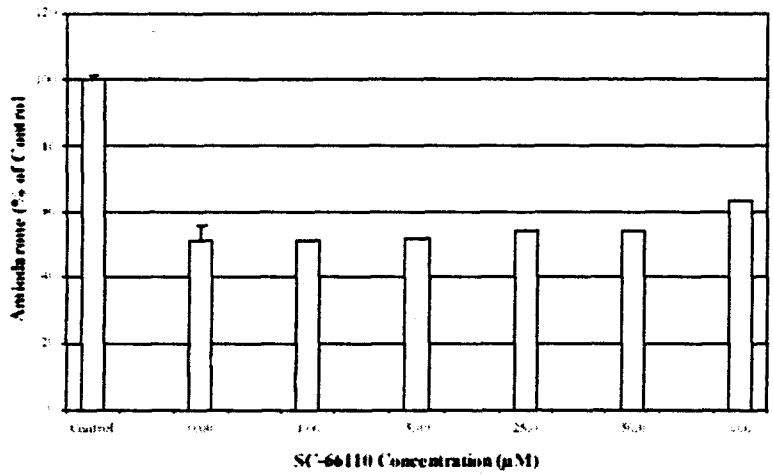
RESULTS:

Effect of Eplerenone on the Disappearance of Amiodarone:

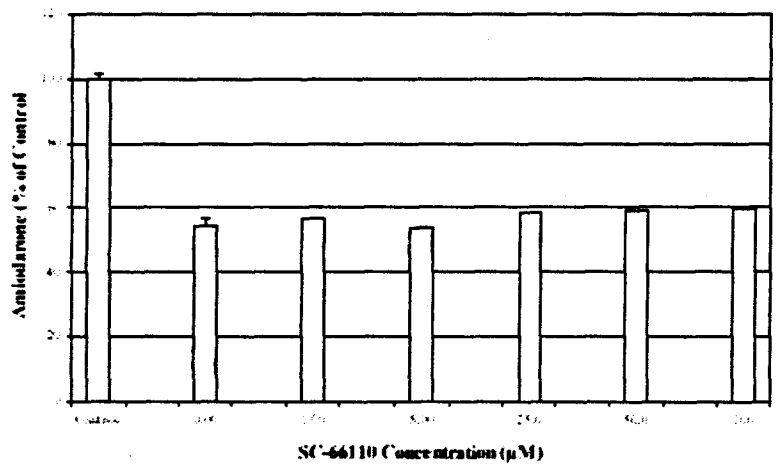
Eplerenone concentrations up to 100 µM did not have an effect on the disappearance of amiodarone although a trend towards higher remaining concentrations of amiodarone with increasing concentrations of eplerenone was apparent. In the control incubations, mean amiodarone concentrations decreased to 51% and 54.3% after 45 minutes incubation when starting amiodarone concentrations were 200 ng/mL or 1000 ng/mL, respectively. This disappearance was dependent on the presence of NADPH thus

indicating substantial P450 metabolism. When amiodarone (200 ng/mL or 1000 ng/mL) were incubated with the highest concentration of eplerenone (100 µM), 63.0% and 59.6%, respectively, remained in the incubation suspensions.

Effect of Eplerenone on the Depletion of Amiodarone (200 ng/mL) In Vitro



Effect of Eplerenone on the Depletion of Amiodarone (1000 ng/mL) In Vitro



Effect of Amiodarone on the Formation of SC-71597:

The Ki estimated for amiodarone inhibition of SC-71597 formation was 46.5 µM using a competitive model of inhibition. The Ki of 46.5 µM substantially exceeds the anticipated plasma concentrations of amiodarone which are typically less than 5 µM. Amiodarone has been noted to inhibit the clearance of other drugs such as warfarin and phenytoin

which are metabolized by CYP2C9. CYP3A4 has been identified as a major enzyme contributing to the formation of SC-71597. The results of the present study suggest that a metabolically based interaction between eplerenone and amiodarone is unlikely.

CONCLUSIONS

Eplerenone concentrations up to 100 μM did not have an effect on the disappearance of amiodarone. The Ki estimated for amiodarone inhibition of SC-71597 formation was 46.5 μM, a value exceeding amiodarone plasma concentrations which are typically less than 5 μM. These data suggest that drug-drug interactions between eplerenone and amiodarone are unlikely in vivo.

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IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND DEXAMETHASONE

Document #: M2000095

OBJECTIVES:

To evaluate the potential for metabolic drug-drug interactions between eplerenone and dexamethasone.

METHODS:

Effect of Eplerenone on the Disappearance of Dexamethasone

The metabolism of dexamethasone was investigated in human liver microsomes, 1.00 mg/mL final concentration. A volume of 25 µL microsomes was added to 450 µL 100 mM potassium phosphate buffer pH 7.4. Eplerenone was added in a volume of 2 µL to final concentrations of 0, 1.00, 5.00, 25.0, 50.0 and 100 µM as appropriate and dexamethasone was added in a volume of 2 µL to reach final concentrations of 100 or 500 ng/mL. The enzymatic reactions were initiated by adding NADPH (25 µL) to a final concentration of 1 µM and the samples were incubated at 37 °C for 45 minutes. The reactions were quenched by the addition of 1.50 mL of 10% cyclohexane in ethyl acetate and the samples were analyzed as described above. To demonstrate that the disappearance of dexamethasone was dependent on the presence of NADPH and therefore a result of P450 metabolism, 6 samples were incubated without NADPH.

Effect of Dexamethasone on the Formation of SC-71597

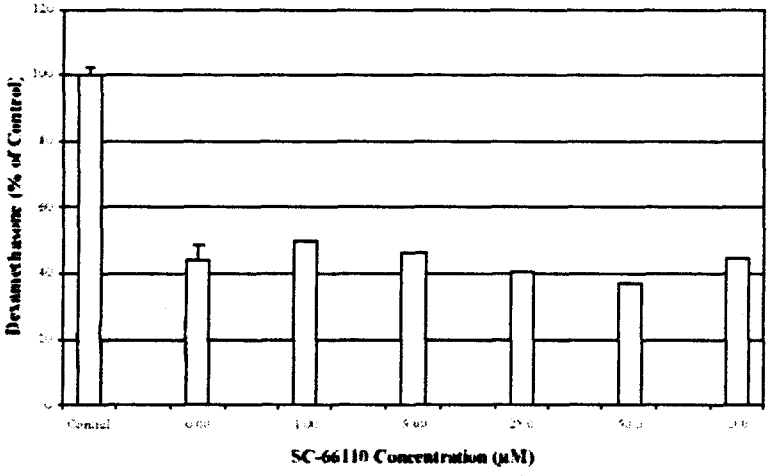
A Ki quantifying inhibition of SC-71597 formation was estimated by incubating 5 Eplerenone (substrate) concentrations with 6 concentrations of dexamethasone (including zero). Human liver microsomes (25 µL) were added to 450 µL of 100 mM potassium phosphate buffer pH 7.4 to achieve a final protein concentration of 0.1 mg/mL. SC-66110 (2 mL in acetonitrile) was added to the appropriate suspensions to achieve the target concentrations of 25.0, 50.0, 100, 200 and 400 µM. Dexamethasone was added to appropriate tubes and the suspensions were allowed to equilibrate for approximately 3 minutes. The concentrations used for dexamethasone were 0, 25.0, 50.0, 100, 200, and 400 µM. The enzymatic reactions were initiated by the addition of NADPH (25 µL) so that the final concentration was 1 mM. Incubations were quenched after 15 minutes by the addition of the extraction solvent ethyl acetate. The samples were injected on to the [redacted] The *m/z* 431→211 product ions of SC-71597 were monitored.

RESULTS:

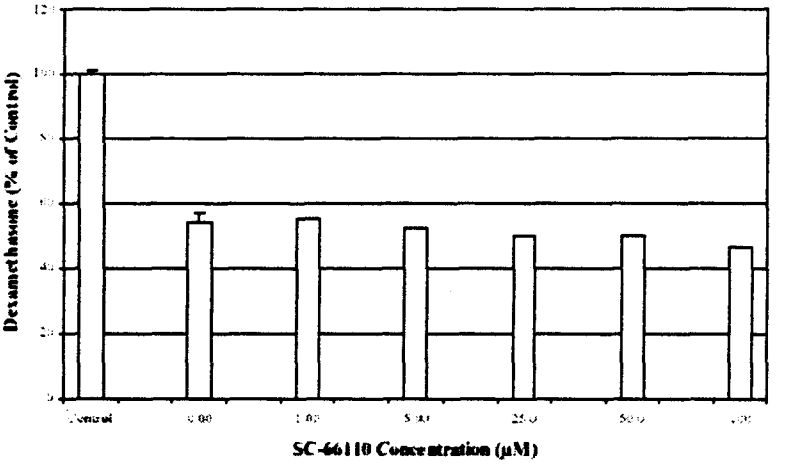
Eplerenone concentrations up to 100 µM had no effect on the disappearance of dexamethasone. When dexamethasone (100 ng/mL or 500 ng/mL) was incubated with the highest concentration of eplerenone, 45.1% and 46.4%, respectively, remained in the

incubation suspensions. In the control incubations, mean dexamethasone concentrations remaining after 45 minutes incubation decreased to 44.2% and 54.1% when starting dexamethasone concentrations were 100 ng/mL or 500 ng/mL, respectively. This disappearance was dependent on the presence of NADPH thus indicating substantial P450 metabolism.

Effect of Eplerenone on the Depletion of Dexamethasone (100 ng/mL) In Vitro



Effect of Eplerenone on the Depletion of Dexamethasone (500 ng/mL) In Vitro



Effect of Dexamethasone on the Formation of SC-71597

The Ki estimated for dexamethasone inhibition of SC-71597 formation was 33.3 μM using a competitive model of inhibition. The Ki value of 33.3 μM exceeds the anticipated plasma concentrations of dexamethasone, therefore the data suggest that inhibition of eplerenone clearance by dexamethasone is unlikely.

CONCLUSIONS

Eplerenone concentrations up to 100 μM had no effect on the disappearance of dexamethasone. The Ki estimated for dexamethasone inhibition of SC-71597 formation was 33.3 μM which exceeds the anticipated plasma concentrations of dexamethasone. Results of the present in vitro study suggest that a drug-drug interaction between eplerenone and dexamethasone is unlikely in vivo.

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IN VITRO DRUG-DRUG INTERACTION STUDIES WITH EPLERENONE AND MEPHOBARBITAL

Document #: M2000331

OBJECTIVES:

1. To assess the potential for eplerenone to affect the in vitro clearance of mephobarbital.
2. To assess the potential for mephobarbital to alter the metabolic formation of SC-71597.

METHODS:

Evaluation of Mephobarbital Disappearance

Metabolism of mephobarbital was investigated in pooled human liver microsomes, 2.00 mg/mL final concentration. A volume of 50 µL microsomes (20.0 mg/mL) was added to 413 µL 100 mM potassium phosphate buffer pH 7.4. Eplerenone was added in a volume of 2 µL to final concentrations of 0, 1.00, 5.00, 25.0, 50.0 and 100 µM. Mephobarbital was added to appropriate tubes in a volume of 12 µL to reach final concentrations of 25 or 75 µg/mL. The enzymatic reactions were initiated after equilibration at 37^0C by adding 25 µL of NADPH regenerating system. The reactions were quenched after 3 hours incubation by addition of 2 mL of ethylacetate and the samples were extracted and analyzed.. To demonstrate that the disappearance of mephobarbital was dependent on the presence of NADPH and therefore a result of P450 metabolism, 6 samples at each mephobarbital concentration were incubated without the regenerating system.

Effect of Mephobarbital on the Formation of SC-71597

Human liver microsomes (25 µL) were added to 450 µL of 100 mM potassium phosphate buffer pH 7.4 to achieve a final protein concentration of 0.100 mg/mL. Eplrerenone (2 µL in acetonitrile) was added to the appropriate suspensions to achieve the target concentrations of 25.0, 50.0, 100, 200 and 400 µM. Mephobarbital was added to appropriate tubes and the suspensions were allowed to equilibrate for approximately 3 minutes. Mephobarbital concentrations used were 0, 50, 100, 250, 375, and 500 µM. The enzymatic reactions were initiated by the addition of NADPH (25 µL) so that the final concentration was 1.00 mM. Incubations were quenched after 15 minutes by the addition of the extraction solvent ethyl acetate. The samples were injected onto the [redacted]
The *m/z* 431→211 product ions of SC-71597 were monitored.

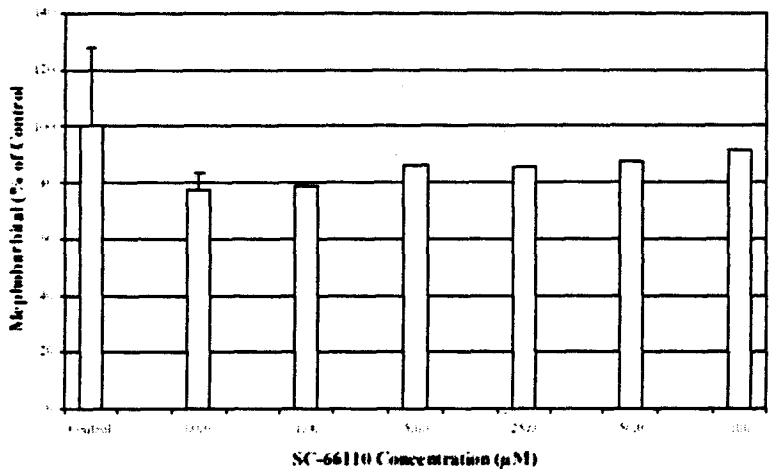
RESULTS:

Evaluation of Mephobarbital Disappearance

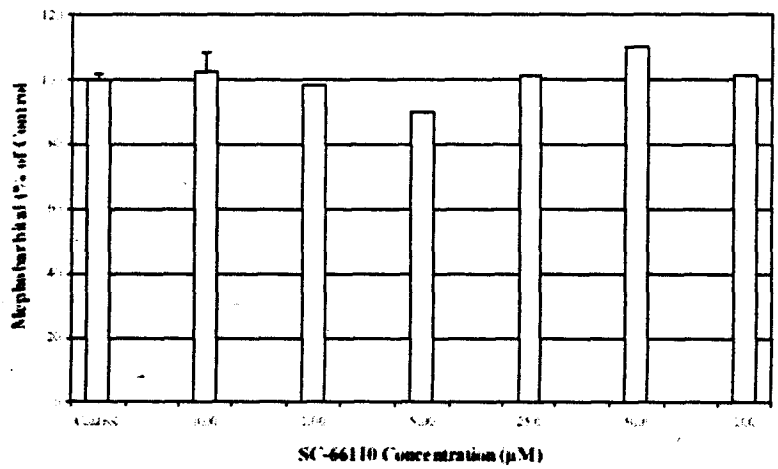
Eplerenone did not have a significant effect on mephobarbital metabolism. There was a trend toward higher concentration of mephobarbital remaining compared to control with

higher concentrations of eplerenone. Mephobarbital decreased by 8.7% in the presence of the highest concentration of eplerenone (100 μM). In control incubations, mean mephobarbital concentration remaining in microsomal suspensions decreased by 22.1% after 3 hours incubation when starting mephobarbital concentration was 25.0 μg/mL. Disappearance was dependent on the presence of the NADPH regenerating system indicating P450 metabolism. This is consistent with roles for CYP2C19 in the hydroxylation of R-mephobarbital and CYP2B6 in the N-demethylation of S-mephobarbital. Disappearance of mephobarbital was not observed when it was included in incubation suspensions at 75 μg/mL. This may suggest that the extent of metabolism occurring was insignificant compared to the starting concentration of mephobarbital.

Effect of Eplerenone on the Depletion of Mephobarbital (25.0 μg/mL) In Vitro



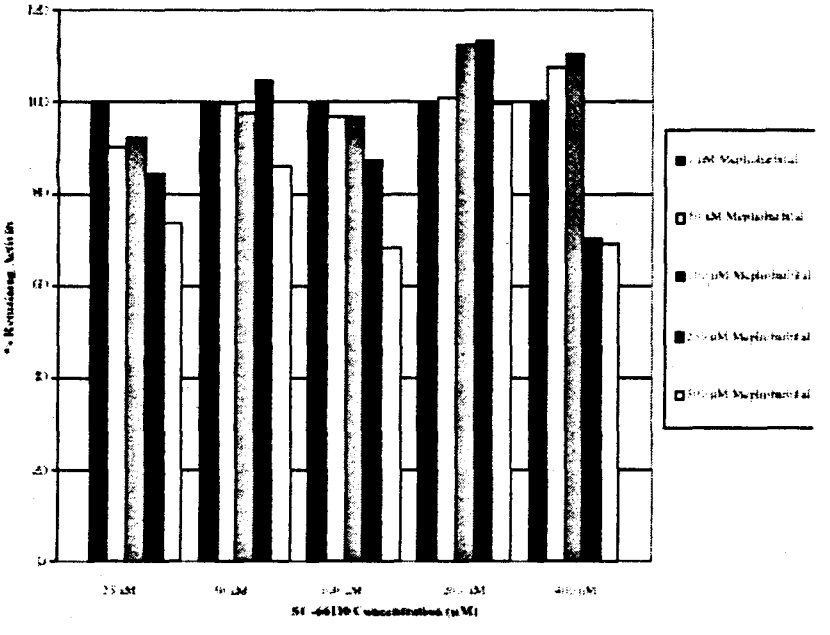
Effect of Eplerenone on the Depletion of Mephobarbital (75.0 μg/mL) In Vitro



Effect of Mephobarbital on the Formation of SC-71597

A Ki was not estimated for mephobarbital inhibition of SC-71597 formation since inhibition did not exceed 40% even at the highest concentration of mephobarbital tested (500 μM). SC-71597 formation velocity was increased in some suspensions containing eplerenone (200 μM) and mephobarbital, particularly those with the higher concentrations of eplerenone. This metabolic activation is most likely due to the cooperative nature of substrate binding to the CYP3A4 enzyme. This phenomenon is documented for CYP3A4. The rate of SC-71597 formation was decreased from the maximal velocity observed when mephobarbital was included in incubation suspensions at the highest concentration (500 μM), however, inhibition exceeding 40% was not observed.

Percent of SC-71597 Formation Remaining in Incubation Suspensions Including Mephobarbital



CONCLUSIONS

Eplerenone did not have a significant effect on mephobarbital metabolism. There was a trend toward higher concentration of mephobarbital remaining compared to control at higher concentrations of eplerenone (100 μM). Disappearance of mephobarbital was not observed when it was included in incubation suspensions at 75 μg/mL. Mephobarbital increased SC-71597 formation velocity in some suspensions, particularly those with the higher concentrations of eplerenone. This metabolic activation is most likely due to the cooperative nature of substrate binding to the CYP3A4 enzyme. This phenomenon is documented for CYP3A4. The rate of SC-71597 formation was decreased from the

maximal velocity observed when mephobarbital was included in incubation suspensions at the highest concentration (500 μM), however, inhibition exceeding 40% was not observed. Results of the present in vitro study suggest a negligible interaction on mephobarbital metabolism in the presence of eplerenone, however, the effect of mephobarbital on eplerenone metabolism is unclear.

APPEARS THIS WAY
ON ORIGINAL

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND PHENYTOIN

Document #: M2000328

OBJECTIVES:

1. To assess the potential for eplerenone to affect the in vitro clearance of phenytoin.
2. To assess the potential for phenytoin to alter the metabolic formation of SC-71597.

METHODS:

Effect of Eplerenone on the Disappearance of Phenytoin

The metabolism of phenytoin was investigated in human liver microsomes, 2 mg/mL final concentration. A volume of 50 µL microsomes was added to 425 µL 100 mM potassium phosphate buffer pH 7.4. Eplerenone was added in a volume of 2 µL to final concentrations of 0, 1.00, 5.00, 25.0, 50.0 and 100 µM as appropriate. In separate tubes, 2.00 mL of sulfaphenazole was added as a positive control inhibitor (final concentration 10 µM). Phenytoin was added to appropriate tubes in a volume of 4 µL to reach final concentrations of 3 or 12 µg/mL. The enzymatic reactions were initiated after equilibration at 37 °C by adding 25 µL of NADPH regenerating system. The reactions were quenched after 3 hours incubation by addition of 3 µL of ethyl acetate. To demonstrate that the disappearance of phenytoin was dependent on the presence of NADPH and therefore a result of P450 metabolism, 6 samples at each phenytoin concentration were incubated without the regenerating system.

Effect of Phenytoin on the Formation of SC-71597

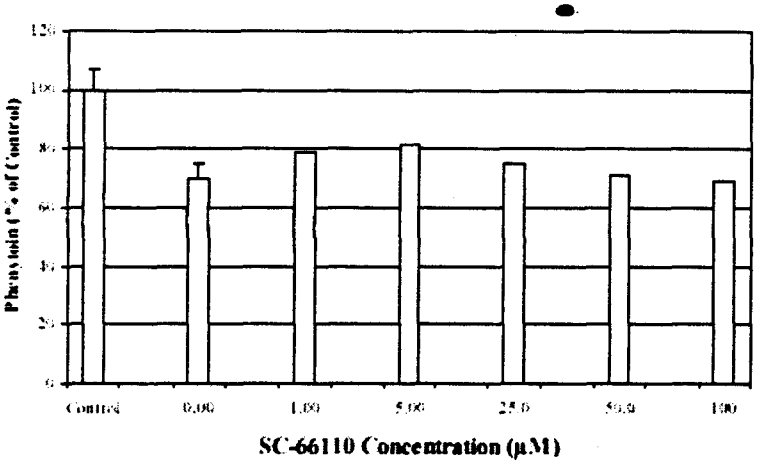
A Ki quantifying inhibition of SC-71597 formation was estimated by incubating 5 eplerenone (substrate) concentrations with 6 concentrations of phenytoin (including zero). Human liver microsomes (25 µL) were added to 450 µL of 100 mM potassium phosphate buffer pH 7.4 to achieve a final protein concentration of 0.100 mg/mL. Eplerenone (2 µL in acetonitrile) was added to the appropriate suspensions to achieve the target concentrations of 25, 50, 100, 200 and 400 µM. Phenytoin was added to appropriate tubes and the suspensions were allowed to equilibrate for approximately 3 minutes. The concentrations used for phenytoin were 0, 27.2, 54.4, 109, 272, and 544 µM. The enzymatic reactions were initiated by the addition of NADPH (25.0 mL) so that the final concentration was 1 µM. Incubations were quenched after 15 minutes by the addition of the extraction solvent ethyl acetate. The samples were injected onto the [redacted] The *m/z* 431→211 product ions of SC-71597 were monitored.

RESULTS:

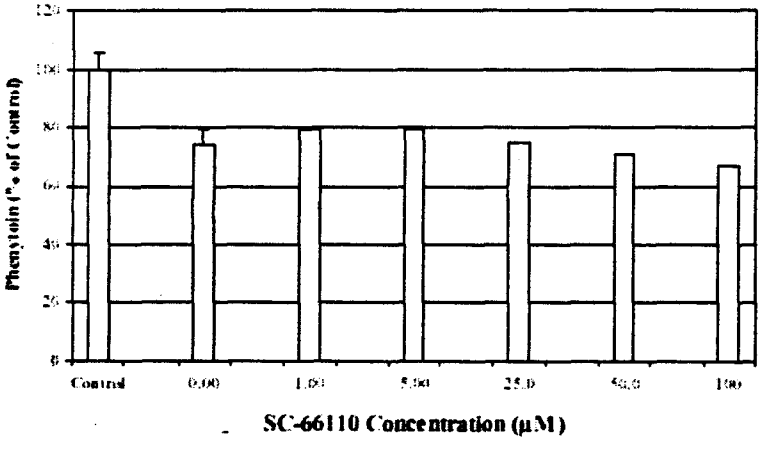
Evaluation of Phenytoin Disappearance

Eplerenone concentrations up to 100 µM did not significantly alter the disappearance of phenytoin. When phenytoin (3 µg/mL or 12 µg/mL) was incubated with the highest concentration of eplerenone (100 µM), 69.2% and 66.8%, respectively, remained in the incubation suspensions. In control incubations, mean phenytoin concentrations remaining in microsomal suspensions decreased to 69.6% and 74.0% after 3 hours incubation when starting phenytoin concentrations were 3 µg/mL or 12 µg/mL, respectively. This disappearance was dependent on the presence of NADPH thus indicating substantial P450 metabolism, R- and S-enantiomers of p-hydroxyphenylhydantoin (p-HPPH) are formed primarily by CYP2C9 and CYP2C19.

Effect of Eplerenone on the Depletion of Phenytoin (3.00 mg/mL) In Vitro



Effect of Eplerenone on the Depletion of Phenytoin (12.0 mg/mL) In Vitro



Effect of Phenytoin on the Formation of SC-71597

The Ki estimated for phenytoin inhibition of SC-71597 formation was 690 µM using a competitive model of inhibition. The Ki value of 690 µM substantially exceeds the

anticipated plasma concentrations of phenytoin, which is approximately 40-60 μM. The results suggest that a metabolically based interaction between eplerenone and phenytoin is unlikely.

CONCLUSIONS

Eplerenone concentrations up to 100 μM did not significantly alter the disappearance of phenytoin. The Ki estimated for phenytoin inhibition of SC-71597 formation was 690 μM which is greater than the anticipated plasma concentrations of phenytoin of 40-60 μM. These results suggest that a metabolically based interaction between eplerenone and phenytoin is unlikely.

APPEARS THIS WAY
ON ORIGINAL

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND PHENACETIN

Document #: M2000326

OBJECTIVES:

1. To assess the potential for eplerenone to affect the CYP1A2-mediated formation of acetaminophen.
2. To assess the potential for phenacetin to alter the metabolic formation of SC-71597.

METHODS:

Effect of Eplerenone on CYP1A2-Mediated Formation of Acetaminophen

The ability of the eplerenone to inhibit the activity of CYP1A2 was evaluated in pooled human liver microsome. Human liver microsomes diluted in 100 mM potassium phosphate buffer at pH 7.4 were fortified with phenacetin to final concentrations of 10.0 or 20.0 µM and eplerenone concentration of 0, 1., 5, 25, 50, and 100 µM and the duplicate suspensions were allowed to equilibrate. Total incubation volumes were 0.5 mL. Metabolic reactions were initiated by the addition of NADPH (1 mM final concentration). Reactions were terminated by the addition of the 0.3 mL acetone. The marker metabolite, acetaminophen (APAP), was quantitated from 1.00 to 200 ng/mL using a validated method. Incubation samples, calibration standards, and quality control samples containing APAP in an incubation buffer matrix (0.5 mL, 100 mM potassium phosphate buffer, pH 7.4, containing 0.100 mg/mL final human liver microsomal protein concentration) were treated by the addition of acetone and the internal standard (N-(4-hydroxyphenyl-2,3,5,6-d_4) acetamide). All samples were injected onto a

Effect of Phenacetin on the Formation of SC-71597

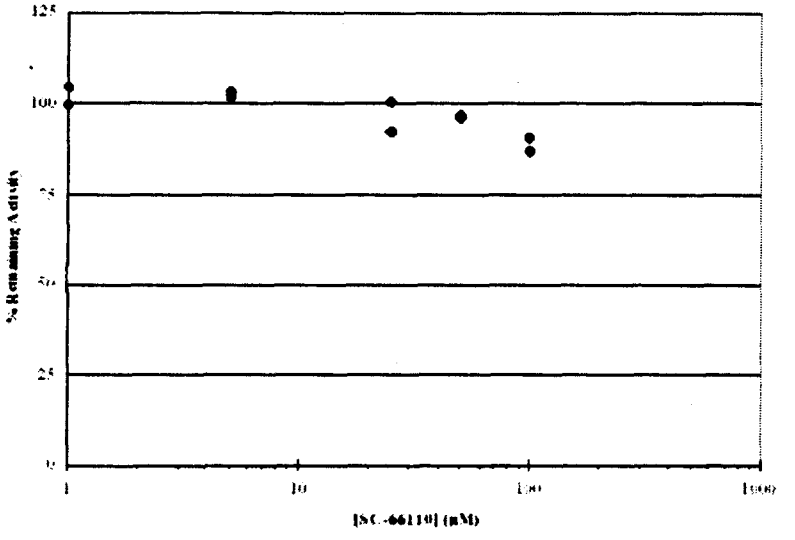
A Ki quantifying inhibition of SC-71597 formation was estimated by incubating 5 eplerenone (substrate) concentrations with 6 concentrations of phenacetin (including zero). Human liver microsomes (25 µL) were added to 450 µL of 100 mM potassium phosphate buffer pH 7.4 to achieve a final protein concentration of 0.1 mg/mL. Eplerenone (2 µL in acetonitrile) was added to the appropriate suspensions to achieve the target concentrations of 25, 50, 100, 200 and 400 µM. Phenacetin was added to appropriate tubes and the suspensions were allowed to equilibrate for approximately 3 minutes. The concentrations used for phenacetin were 0, 25, 50, 100, 200, and 400 µM. The enzymatic reactions were initiated by the addition of NADPH (25.0 µL) so that the final concentration was 1.00 mM. Incubations were quenched after 15 minutes by the addition of the extraction solvent ethyl acetate. The samples were injected onto the ——— and the *m/z* 431→211 product ions of SC-71597 were monitored.

RESULTS:

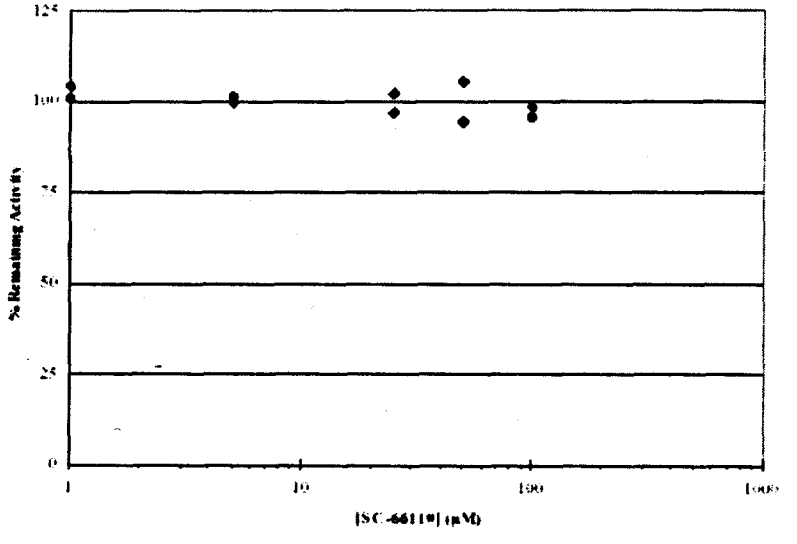
Effect of Eplerenone on CYP1A2-Mediated Formation of Acetaminophen

Incubation of eplerenone, at concentrations up to 100 μM, did not inhibit CYP1A2-mediated formation of acetaminophen when phenacetin was incubated at either concentration (10.0 μM or 20.0 μM). These data suggest that eplerenone will not inhibit the clearance of phenacetin or other substrates of CYP1A2.

Effect of Eplerenone on Formation of Acetaminophen When Incubated with Phenacetin (10.0 μM) In Vitro



Effect of Eplerenone on Formation of Acetaminophen When Incubated with Phenacetin (20.0 μM) In Vitro



Effect of Phenacetin on the Formation of SC-71597

The Ki estimated for phenacetin inhibition of SC-71597 formation was 470 μM using a mixed model of inhibition. The Ki value of 470 μM exceeds the plasma concentrations of phenacetin obtained following administration of therapeutic doses (<2 μg/mL following an oral dose of 900 mg). The present results suggest that a metabolically based interaction between eplerenone and phenacetin is unlikely.

CONCLUSIONS

Incubation of eplerenone, at concentrations up to 100 μM, did not inhibit CYP1A2-mediated formation of acetaminophen when phenacetin was incubated at either concentration (10 μM or 20 μM). The Ki estimated for phenacetin inhibition of SC-71597 formation was 470 μM using a mixed model of inhibition. The Ki value of 470 μM exceeds the plasma concentrations of phenacetin obtained following administration of therapeutic doses (<2 μg/mL following an oral dose of 900 mg). The present results suggest that a metabolically based interaction between eplerenone and phenacetin is unlikely.

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND DEXTROMETHORPHAN

Document #: M2000361

OBJECTIVES:

1. To assess the potential for eplerenone to affect the CYP2D6-mediated formation of dextrorphan.
2. To assess the potential for dextromethorphan to alter the metabolic formation of SC-71597.

METHODS:

Effect of Eplerenone on CYP2D6-Mediated Formation of Dextrorphan

The ability of the SC-66110 to inhibit the activity of CYP2D6 was evaluated in pooled human liver microsomes.Human liver microsomes diluted in 100 mM potassium phosphate buffer at pH 7.4 were fortified with dextromethorphan to final concentrations of 5.00 or 20.0 µM and eplerenone concentrations of 0, 1, 5, 25, 50 and 100 µM and the duplicate suspensions were allowed to equilibrate. Total incubation volumes were 0.5 mL. Metabolic reactions were initiated by the addition of NADPH (1 mM final concentration). Reactions were terminated by the addition of the 0.3 mL acetone. Samples were injected into ——— and peak areas of the m/z 258→157 product ions of DRR were measured.

Effect of Dextromethorphan on the Formation of SC-71597

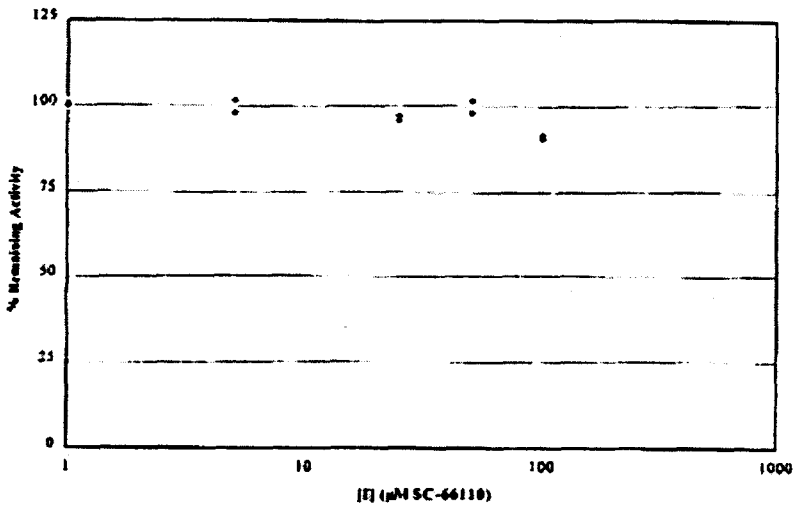
A Ki quantifying inhibition of SC-71597 formation was estimated by incubating 5 eplerenone (substrate) concentrations with 6 concentrations of dextromethorphan (including zero).Human liver microsomes (25 µL) were added to approximately 425 µL of 100 mM potassium phosphate buffer pH 7.4 to achieve a final protein concentration of 0.1 mg/mL. Eplerenone (2 µL in acetonitrile) was added to the appropriate suspensions to achieve the target concentrations of 25.0, 50.0, 100, 200 and 400 µM. Dextromethorphan was added to appropriate tubes and the suspensions were allowed to equilibrate for approximately 3 minutes. The concentrations used for dextromethorphan were 0, 150, 250, 500, 1000, and 2000 µM. The enzymatic reactions were initiated by the addition of NADPH (25 µL) so that the final concentration was 1 mM. Incubations were quenched after 15 minutes by the addition of the extraction solvent ethyl acetate. The samples were injected onto the ——— The *m/z* 431→211 product ions of SC-71597 were monitored.

RESULTS:

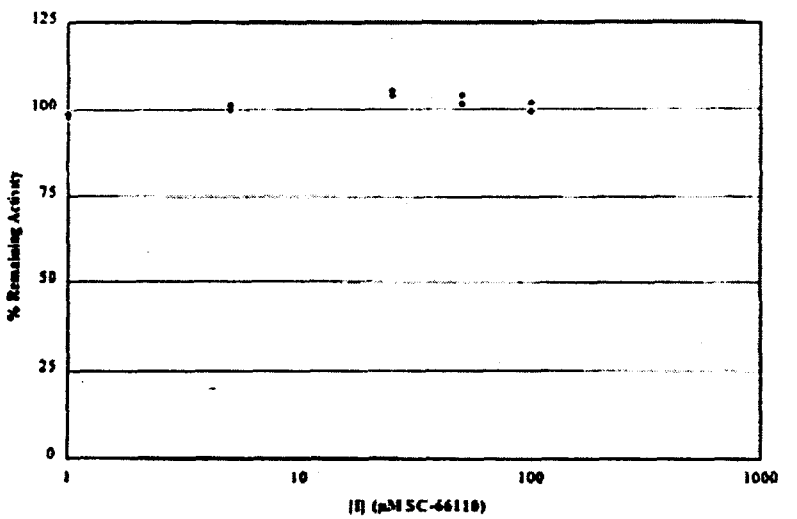
Effect of Eplerenone on CYP2D6-Mediated Formation of Dextrorphan

Incubation of eplerenone at concentrations up to 100 μM did not result in inhibition of CYP2D6-mediated formation of dextrorphan when dextromethorphan was incubated at either concentration 5 μM or 20 μM), suggesting that eplerenone will not inhibit the clearance of dextromethorphan or other substrates of CYP2D6 in vivo.

Effect of Eplerenone on the Formation of Dextrorphan When Incubated with Dextromethorphan (5.00 μM) In Vitro



Effect of Eplerenone on the Formation of Dextrorphan When Incubated with Dextromethorphan (20.0 μM) In Vitro



Effect of Dextromethorphan on the Formation of SC-71597

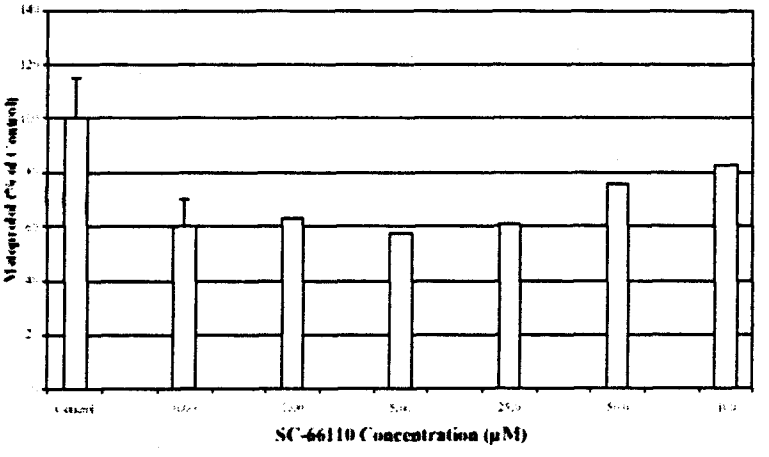
Dextromethorphan inhibited the formation of SC-71597 in a concentration dependent manner. The Ki estimated for dextromethorphan inhibition of SC-71597 formation was 360 μM using a competitive model of inhibition. The Ki value of 360 μM exceeds the plasma concentrations of dextromethorphan administered at normal doses. These results suggest that a metabolically based interaction between eplerenone and dextromethorphan is unlikely.

CONCLUSIONS

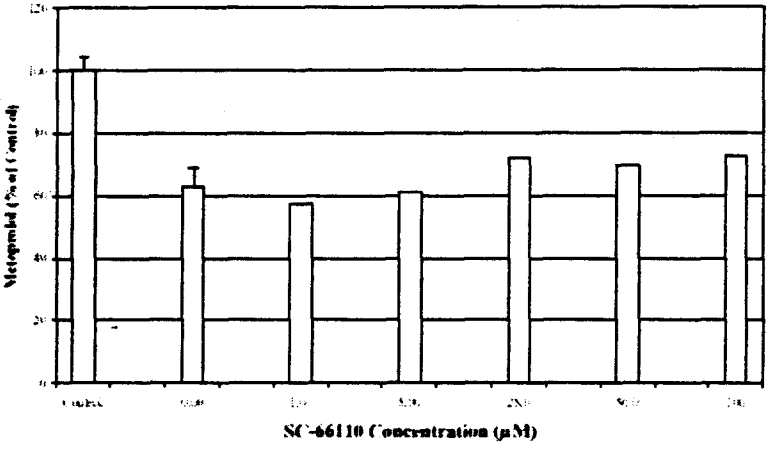
Incubation of eplerenone at concentrations up to 100 μM did not result in inhibition of CYP2D6-mediated formation of dextrorphan when dextromethorphan was incubated at either concentration 5 μM or 20 μM), suggesting that eplerenone will not inhibit the clearance of dextromethorphan or other substrates of CYP2D6 in vivo. The Ki estimated for dextromethorphan inhibition of SC-71597 formation was 360 μM, which concentration exceeds the plasma concentrations obtained following normal doses of dextromethorphan. These results suggest that a metabolically based in vivo interaction between eplerenone and dextromethorphan is unlikely.

Eplerenone at concentrations up to 100 μM decreased the disappearance of metoprolol. A trend towards higher remaining concentrations of metoprolol with increasing concentrations of eplerenone was observed. When metoprolol (5 μg/mL or 10 μg/mL) was incubated with the highest concentration of eplerenone (100 μM), 82.9% and 72.6%, respectively, metoprolol was remaining compared to control incubations, where 60% and 62.6% was remaining after 2 hours of incubation when starting metoprolol concentrations were 5 μg/mL or 10 μg/mL, respectively. This disappearance was dependent on the presence of the NADPH regenerating system thus indicating substantial P450 metabolism.

Effect of Eplerenone on the Depletion of Metoprolol (5 mg/mL) In Vitro



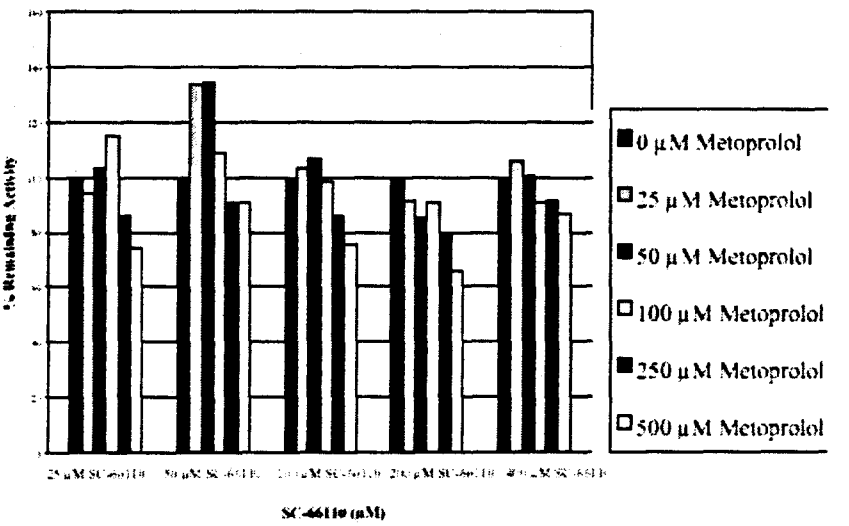
Effect of Eplerenone on the Depletion of Metoprolol (10 mg/mL) In Vitro



Effect of Metoprolol on the Formation of SC-71597

The velocities of SC-71597 formation observed were marginally decreased when metoprolol was included at higher concentrations; i.e. above 100 µM. Inhibition exceeding 40% was not observed even at the highest concentration of metoprolol (500 µM). Therefore, an IC50 could not be estimated. The decrease in SC-71597 formation at high concentration of metoprolol ranged between 15% and 40% compared to controls, while at lower concentrations metoprolol increased the formation velocity especially at eplerenone concentration of 50 µM.

Effect of Metoprolol on SC-71597 Formation at Five Eplerenone Concentrations



CONCLUSIONS

Eplerenone at concentrations up to 100 µM decreased the disappearance of metoprolol. A trend towards higher remaining concentrations of metoprolol with increasing concentrations of eplerenone was observed. When metoprolol (5 µg/mL or 10 µg/mL) was incubated with the highest concentration of eplerenone (100 µM), 82.9% and 72.6%, respectively, was remaining compared to control incubations, where mean metoprolol concentrations remaining in incubation suspensions decreased to 60% and 62.6% after 2 hours of incubation when starting metoprolol concentrations were 5 µg/mL or 10 µg/mL, respectively. Metoprolol decreased the velocities of SC-71597 formation at higher concentrations; i.e. above 100 µM. Inhibition exceeding 40% was not observed even at the highest concentration of metoprolol (500 µM). The decrease in SC-71597 formation at high concentration of metoprolol ranged between 15% and 40% compared to controls, while at lower concentrations metoprolol increased the formation velocity especially at eplerenone concentration of 50 µM. At therapeutic doses, no interaction is anticipated between eplerenone and metoprolol.

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND TOLBUTAMIDE

Document #: M20000329

OBJECTIVES:

1. To assess the potential for eplerenone to affect the in vitro CYP2C9-mediated formation of 4-hydroxytolbutamide.
2. To assess the potential for tolbutamide to alter the metabolic formation of SC-71597.

METHODS:

Effect of Eplerenone on CYP2C9-Mediated Formation of 4-Hydroxytolbutamide

The ability of the eplerenone to inhibit the activity of CYP2C9 was evaluated in pooled human liver microsomes. Human liver microsomes diluted in 100 mM potassium phosphate buffer at pH 7.4 were fortified with tolbutamide to final concentrations of 140 or 280 µM and eplerenone concentrations were 0, 1, 5, 25, 50 and 100 µM and the duplicate suspensions were allowed to equilibrate. Total incubation volumes were 0.5 mL. Metabolic reactions were initiated by the addition of NADPH (1 mM final concentration). Reactions were terminated by the addition of the 0.3 mL acetone. The marker metabolite, 4-hydroxytolbutamide (HTB), was quantitated from 10.0 to 2000 ng/mL. Samples were injected onto [redacted] and peak areas of the m/z 285→186 product ion of HTB were measured.

Effect of Tolbutamide on the Formation of SC-71597

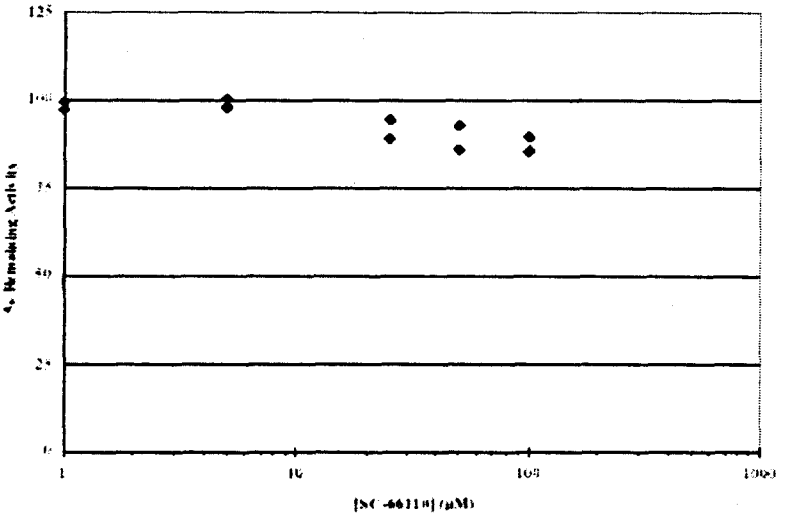
Tolbutamide inhibition of SC-71597 formation was estimated by incubating 5 eplerenone (substrate) concentrations with 6 concentrations of tolbutamide (including zero). Human liver microsomes (25 µL) were added to 450 µL of 100 mM potassium phosphate buffer pH 7.4 to achieve a final protein concentration of 0.1 mg/mL. Eplerenone (2.00 µL in acetonitrile) was added to the appropriate suspensions to achieve the target concentrations of 25.0, 50.0, 100, 200 and 400 µM. Tolbutamide was added to appropriate tubes and the suspensions were allowed to equilibrate for approximately 3 minutes. Tolbutamide concentrations used were 0, 100, 200, 350, 750, and 1000 µM. The enzymatic reactions were initiated by the addition of NADPH (25 µL) so that the final concentration was 1.00 mM. Incubations were quenched after 15 minutes by the addition of the extraction solvent ethyl acetate. The samples were injected onto the [redacted] and peak areas of *m/z* 431→211 product ions of SC-71597 were monitored.

RESULTS:

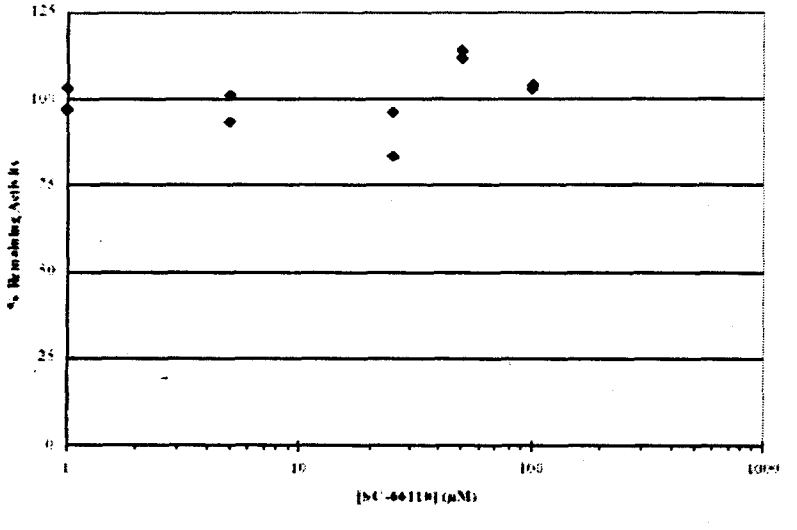
Effect of Eplerenone on CYP2C9-Mediated Formation of 4-hydroxytolbutamide

Incubation of eplerenone concentrations up to 100 μM did not result in inhibition of CYP2C9-mediated formation of 4-hydroxytolbutamide when tolbutamide was incubated at either 140 μM or 280 μM concentrations. These results suggest that eplerenone does not inhibit CYP2C9 and is not likely to inhibit the clearance of tolbutamide or other substrates of CYP2C9.

Effect of Eplerenone on the Formation of 4-hydroxytolbutamide When Incubated with Tolbutamide (140 μM) In Vitro



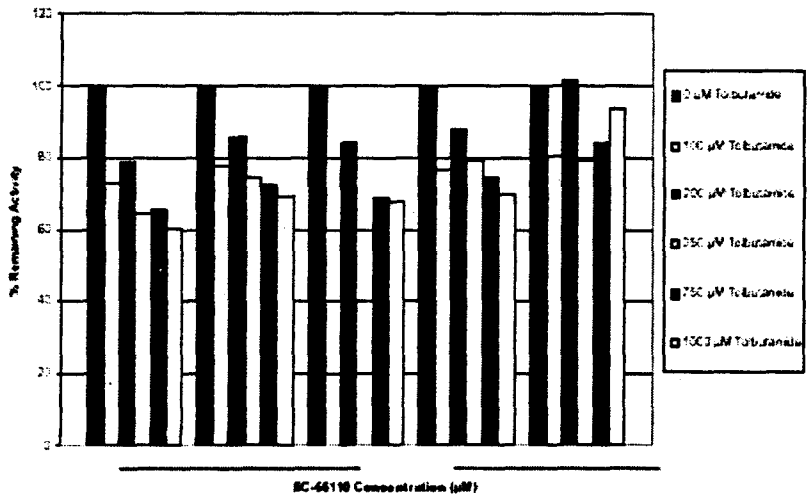
Effect of Eplerenone on the Formation of 4-hydroxytolbutamide When Incubated with Tolbutamide (280 μM) In Vitro



Effect of Tolbutamide on the Formation of SC-71597

The velocity of SC-71597 formation was decreased in a concentration dependent manner by tolbutamide when eplerenone was incubated at 25, 50, 100 or 200 μM. However, this trend was not consistent at the highest substrate (eplerenone) concentration (400 μM). Inhibition exceeding 40% was not observed at any concentration of tolbutamide therefore the Ki value was not estimated.

The Effect of Tolbutamide on the Formation of SC-71597



CONCLUSIONS

Incubation of eplerenone concentrations up to 100 μM did not result in inhibition of CYP2C9-mediated formation of 4-hydroxytolbutamide when tolbutamide was incubated at either 140 μM or 280 μM concentrations. These results suggest that eplerenone might not inhibit CYP2C9. The velocity of SC-71597 formation was decreased in a concentration dependent manner by tolbutamide when eplerenone was incubated at 25, 50, 100 or 200 μM. However, this trend was not consistent at the highest substrate (eplerenone) concentration (400 μM). Inhibition did not exceed 40% at the highest concentration of tolbutamide (1000 μM).

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND EITHER KETOCONAZOLE OR FLUCONAZOLE

Document #: M20978362

OBJECTIVES:

To evaluate the potential for metabolically based drug-drug interactions between eplerenone and known inhibitors of CYP3A4, either ketoconazole or fluconazole.

METHODS:

Effect of Ketoconazole or Fluconazole on the Formation of SC-71597

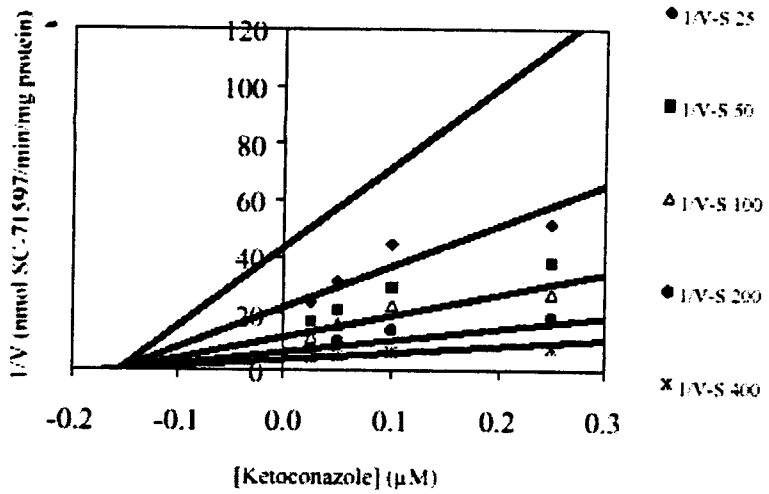
Human liver microsomes (25 µL) were added to 450 µL of 100 mM potassium phosphate buffer pH 7.4 to achieve a final protein concentration of 0.1 mg/mL. Eplerenone (2 µL in acetonitrile) was added to the appropriate suspensions to achieve the target concentrations of 25.0, 50.0, 100, 200 and 400 µM. Ketoconazole or fluconazole was added in separate experiments to appropriate tubes and the suspensions were allowed to equilibrate for approximately 3 minutes. The concentrations of ketoconazole used were 0, 0.025, 0.05, 0.1, 0.25 and 0.5 µM and concentration of fluconazole were 0, 10, 25, 50, 100, and 250 µM. The enzymatic reactions were initiated by the addition of NADPH (25.0 mL) so that the final concentration was 1.00 mM. The enzymatic reactions were quenched after 15 minutes by the addition of the extraction solvent ethyl acetate. The samples were injected onto the [redacted] and peak areas measured for *m/z* 431→211 product ions of SC-71597.

RESULTS

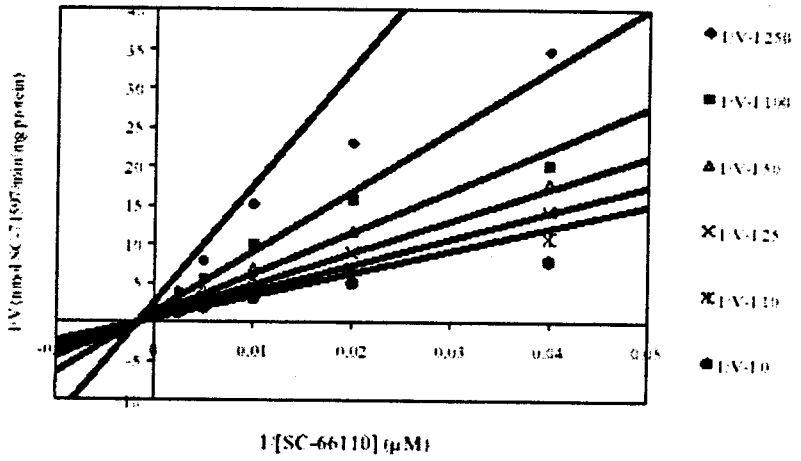
Effect of Ketoconazole or Fluconazole on the Formation of SC-71597

The Ki estimated for ketoconazole inhibition of SC-71597 formation was 0.16 µM using a non-competitive model of inhibition. The data for fluconazole inhibition of SC-71597 formation most closely fit a noncompetitive model with an estimated Ki of 59 µM. Ketoconazole was a more potent inhibitor of SC-71597 formation compared to fluconazole. Some curvature is noted in the observed data which was explained by cooperativity of substrate binding, a known phenomenon of CYP3A4. Data from incubation samples with the highest concentration of ketoconazole were not included in the estimation of Ki in order to minimize this effect. Ketoconazole, fluconazole, and other azole antifungal drugs such as miconazole and itraconazole are known to decrease the clearance of drugs metabolized by CYP3A4 in vivo. The results of the present study suggests that the clearance of eplerenone through metabolic pathways involving the formation of SC-71597 is likely to be decreased when coadministered with ketoconazole or fluconazole.

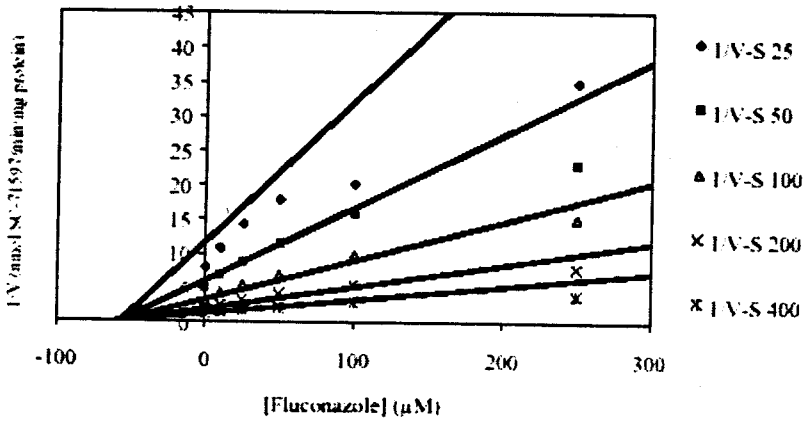
Lineweaver-Burke Plot of Fitted Lines Over Observed Data for Ketoconazole Inhibition of SC-71597 Formation



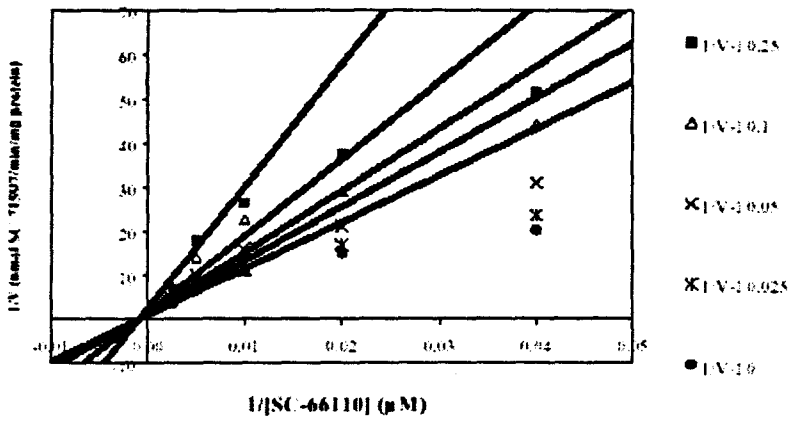
Dixon Plot of Fitted Lines Over Observed Data for Ketoconazole Inhibition of SC-71597 Formation



Lineweaver-Burke Plot of Fitted Lines Over Observed Data for Fluconazole Inhibition of SC-71597 Formation



Dixon Plot of Fitted Lines Over Observed Data for Fluconazole Inhibition of SC-71597 Formation



CONCLUSIONS

Ketoconazole inhibited the formation of SC-71597 with a Ki value of 0.16 µM. Similarly, fluconazole inhibited the formation of SC-71597 with a Ki value of 59 µM. Comparison of Ki values for the inhibitors suggest that ketoconazole is a more potent inhibitor of SC-71597 formation compared to fluconazole. The results of this study suggest that the clearance of eplerenone through metabolic pathways involving the formation of SC-71597 is likely to be decreased when coadministered with the ketoconazole and fluconazole.

SC-66110 (EPLERENONE) INTERACTION STUDIES IN HUMAN LIVER MICROSOMES

Document #: M2099147

OBJECTIVES:

1. To assess the potential for concomitant medications to alter the metabolic clearance of eplerenone through formation of SC-71597.
2. To assess the potential for eplerenone to affect the clearance of drugs administered concomitantly.

METHODS:

19 compounds, having significant structural diversity, were selected from several therapeutic classes. The drugs were examined as potential interacting drugs partially because many of them are substrates of CYP3A4. Each compound was examined, using human liver microsomes, for its potential to affect the formation velocity (v) of SC-71597 from eplerenone. Conversely, the effect of eplerenone on the disappearance of each compound from microsomal suspensions was evaluated by monitoring the concentration of the interacting drug in microsomal suspensions incubated in the absence or presence of eplerenone.

Determination of Inhibition Constants

A Ki quantifying inhibition of SC-71597 formation from eplerenone was estimated for each of the interacting drugs by incubating in duplicate 5 eplerenone (substrate) concentrations with 6 inhibitor concentrations (including zero). Briefly, human liver microsomes (25 µL) were added to 450 µL of 100 mM potassium phosphate buffer pH 7.4 to achieve a final protein concentration of 0.1 mg/mL. Eplerenone (2 µL in acetonitrile:water) was added to the appropriate suspensions to achieve the target concentrations of 25, 50, 100, 200 and 400 µM. The inhibitor (interacting drug) was added to appropriate tubes and the suspensions were allowed to equilibrate for approximately 3 minutes. The concentrations used for each interacting drug (presented in Table 2) were based on the anticipated Ki as estimated from a review of the literature and spanned a range from approximately 25% to 500% of the expected Ki. The enzymatic reactions were initiated by the addition of NADPH (25 mL) so that the final concentration was 1 mM. Incubations were quenched after 15, 20, or 30 minutes by the addition of the extraction solvent ethyl acetate. The samples were injected onto the [redacted] and peak areas for *m/z* 431→211 product ions of SC-71597 were monitored.

RESULTS

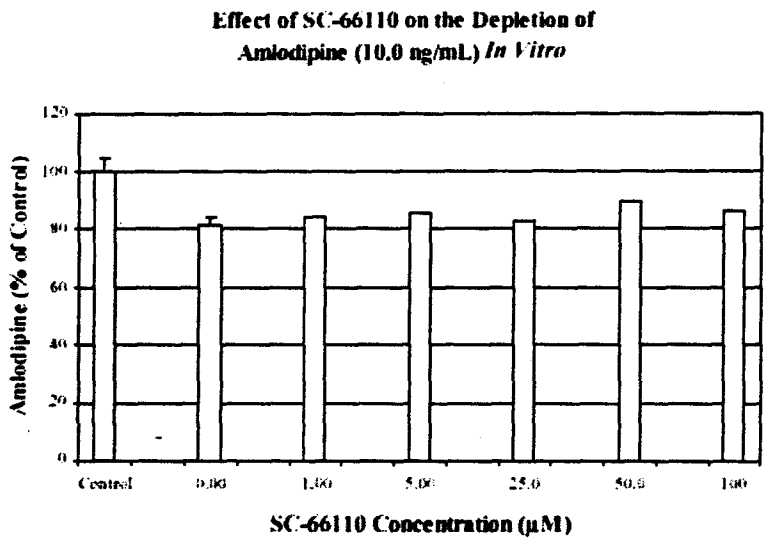
The following table lists the concentrations of the 19 drugs used in the inhibition study and their method of inhibition.

Table 1. Concentrations of Interacting Drugs Incubated with Eplerenone and the Experimental Results

Drug	Concentrations included in incubations (μM)	Ki Estimated (μM)	Type of Inhibition
Amlodipine	0,5,125,250,500,1000	412	Competitive
Astemizole	0,0.1,1,10,30,100	2.72	Competitive
Cisapride	0,4,10,20,40,100	2.90	Competitive
Cyclosporine	0,5,10,16,30,50	1.24	Non-Competitive
Diazepam	0, 35, 50, 80, 140, 250	80.0	Competitive
Digoxin	0,5,10,25,50,75	No Inhibition observed.	
Erythromycin	0,5,10,20,50,100	9.47	Competitive
17α-Ethinylestradiol	0,2.5,5,10,25,50	19.5	Non-Competitive
Fluoxetine	0,15,30,50,125,250	17.6	Linear Mixed
Lovastatin	0,2,5,10,25,50	11.9	Competitive
Methylprednisolone	0,50,100,200,400,800	124	Non-Competitive
Midazolam	0,2.5,5,10,20,50	8.10	Non-Competitive
Nifedipine	0,20,40,70,110,200	21.8	Non-Competitive
Saquinavir	0,0.35,0.7,1.2,2,3.5	0.546	Linear Mixed
Simvastatin	0,2.5,5,10,20,50	6.23	Competitive
Triazolam	0,40,75,120,200,375	408	Competitive
Verapamil	0,12.5,25,40,70,125	13.3	Competitive
(R+)-Warfarin	0,50,100,200,400,800	784	Competitive
(S-)-Warfarin	0,50,100,200,400,800	750	Competitive

Potential for Interaction with Amlodipine

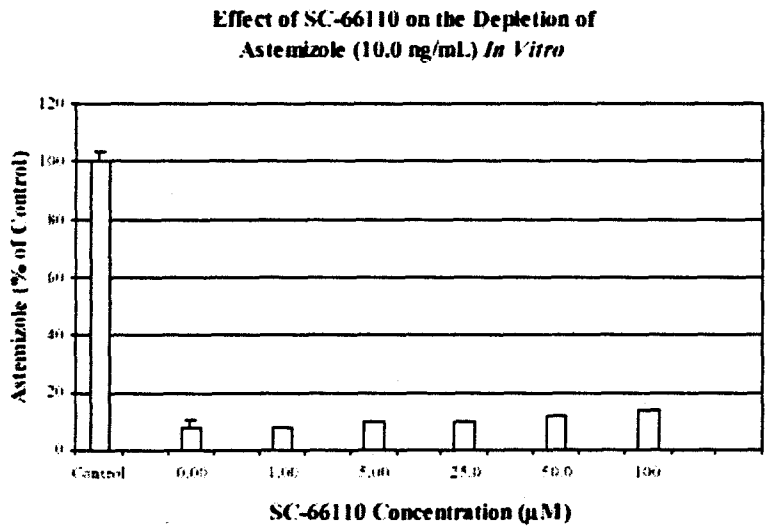
Eplerenone at concentrations up to 100 μM did not affect in vitro metabolism of amlodipine. In control incubations, about 20% of amlodipine was metabolized in the microsomal system during the 30 min incubation. Eplerenone is not expected to affect the metabolism of amlodipine in vivo.



Amlodipine inhibited SC-71597 formation from eplerenone. Using a competitive model of inhibition, the estimated Ki was 412 μM. The maximal plasma concentration (Cmax) reported following a single oral 5 mg dose of amlodipine of 3.1 ± 0.6 ng/mL equivalent

to ~7.6 nM is lower than the estimated Ki for inhibition of SC-71597 formation. The results suggest that changes in eplerenone and amlodipine pharmacokinetics are unlikely to occur when both drugs are co-administered in vivo.

Potential for Interaction with Astemizole

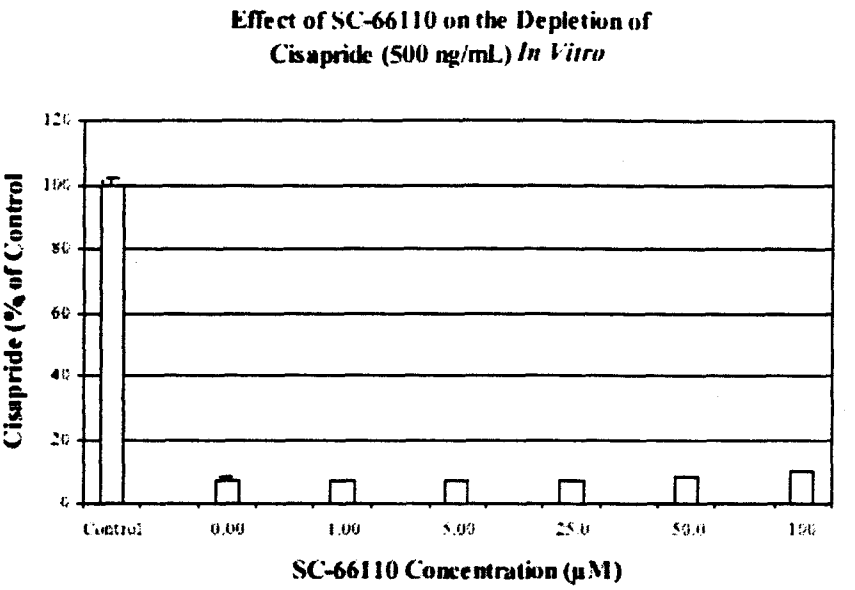


A concentration dependent decrease in the extent of depletion of astemizole was observed. In the presence of the highest concentration of eplerenone (100 µM) 16.0% and 13.7% of astemizole were remaining. In the control incubations, only 8.58% and 7.96% of the respective starting concentrations of astemizole remained following a 20 min incubation in microsomes indicating extensive metabolism by NADPH dependent mechanisms. The concentrations of eplerenone that demonstrated substantial inhibition exceed the anticipated plasma concentrations following therapeutic doses of eplerenone, therefore, clinically relevant effect of eplerenone on astemizole metabolism is not expected.

Astemizole competitively inhibited SC-71597 formation with a Ki estimate by nonlinear regression of 2.72 µM. Clinical interactions resulting in decreased eplerenone clearance are not expected in vivo since concentrations of astemizole are less than 20 nM following chronic daily administration of 10 mg doses.

Potential for Interaction with Cisapride

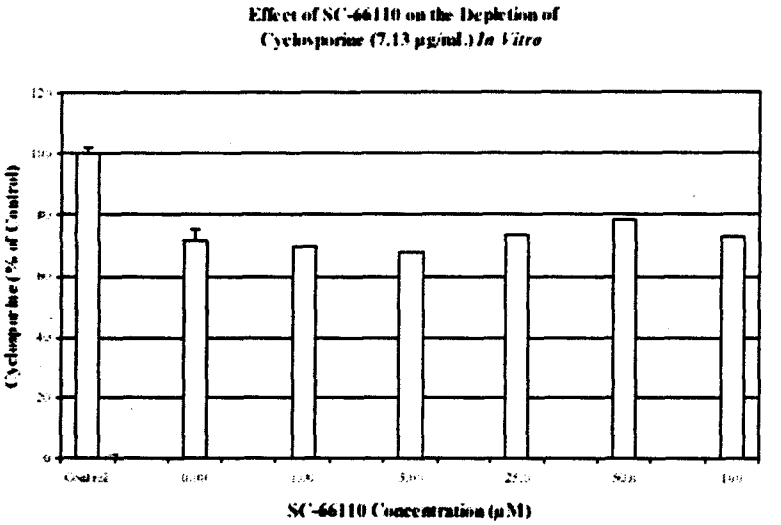
Eplerenone concentrations inhibited cisapride disappearance in a concentration dependent manner. Eplerenone concentrations of 100 µM resulted in 9.63% cisapride remaining, while in control incubations with an initial concentration of 100 ng/mL, cisapride was completely depleted and with an initial concentration of 500 ng/mL, 7.07% cisapride remained after 30 min incubation. The concentration of eplerenone demonstrating inhibition in this experiment exceeds the anticipated plasma concentrations (<5 µM) after 100 mg QD eplerenone.



Cisapride competitively inhibited formation of SC-71597 from eplerenone. The nonlinear estimate of Ki was 2.90 µM. The potential for clinical interaction resulting from cisapride inhibition, however, appears to be low since maximal cisapride plasma concentrations are less than 200 nM following a single oral 10 mg dose.

Potential for Interaction with Cyclosporine

Eplerenone concentrations up to 100 µM did not alter the disappearance of cyclosporin. In control incubations, percent cyclosporine remaining in microsomal incubations was reduced to 46.5% and 71.7% of the low and high starting concentrations, respectively, suggesting P450 dependent microsomal metabolism. These results indicate that eplerenone does not have a significant effect on the metabolism of cyclosporine and that no important changes in the clearance of cyclosporine are anticipated in vivo as a result



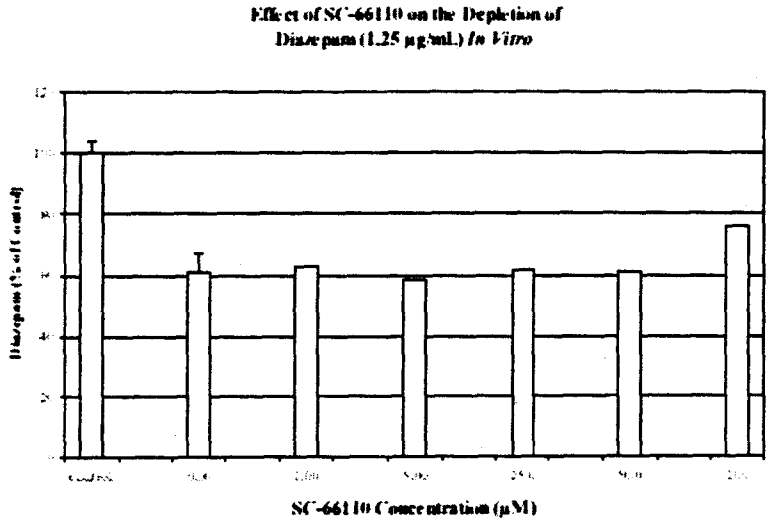
of eplerenone inhibition of metabolism.

Cyclosporine was a potent inhibitor of SC-71597 formation with a Ki of 1.24 µM. The data fit a noncompetitive model of inhibition. The potential exists for significant changes in eplerenone clearance due to cyclosporine inhibition of SC-71597 formation since

maximal plasma concentrations of cyclosporine may reach or exceed its estimated Ki in patients. Clinically significant interactions between cyclosporine and eplerenone are expected.

Potential for Interaction with Diazepam

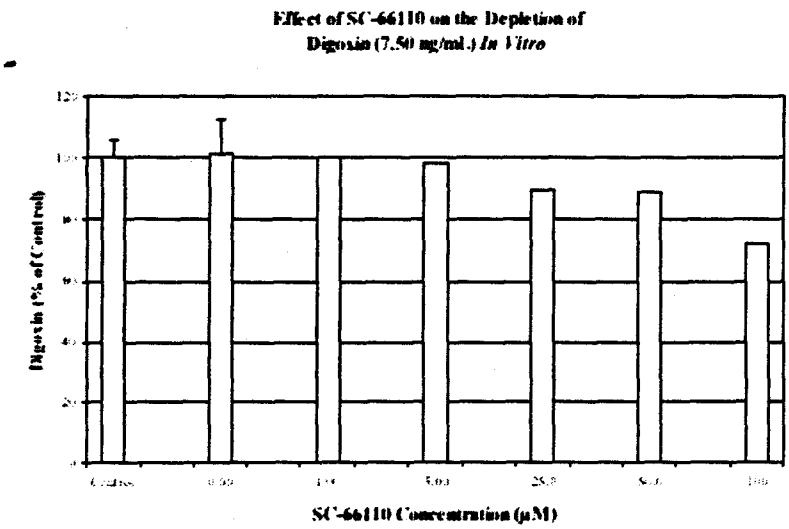
Eplerenone at concentrations up to 50 μM did not affect the disappearance of diazepam, however, at the highest eplerenone concentration of 100 μM the disappearance of diazepam decreased from about 39% to 24%. In control incubations, optimized with higher concentration of microsomal protein and longer incubation time, approximately 40% of the diazepam was metabolized. The concentration of eplerenone that demonstrated inhibition in this experiment is substantially higher than anticipated plasma concentrations and no significant clinical interactions are anticipated.

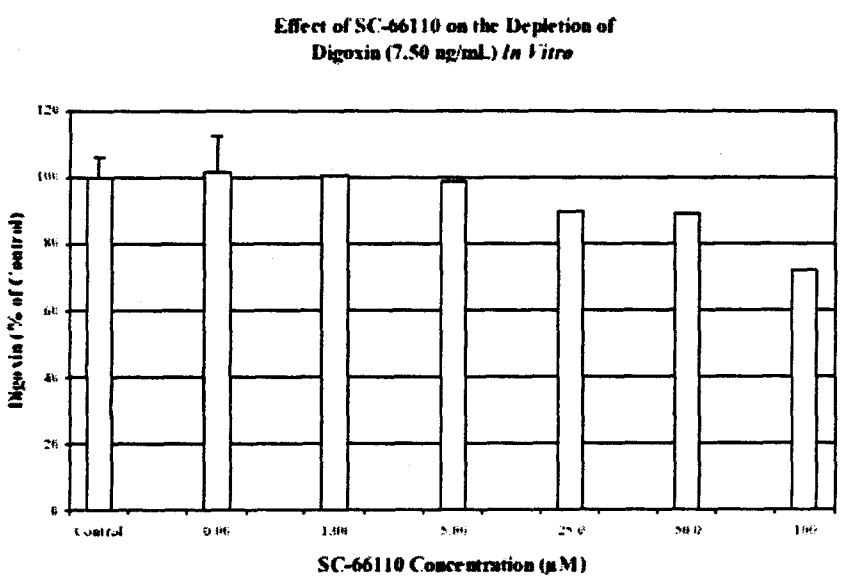


Diazepam inhibited the formation of SC-71597 with Ki of 80.0 μM estimated from competitive model of inhibition. Diazepam concentrations reached 340 ± 57 ng/mL which is equivalent to ~1.2 μM) in patients after a single 10 mg oral dose. The results indicate that diazepam will not have a significant effect on the clearance of eplerenone.

Potential for Interaction with Digoxin

A significant reduction of digoxin concentrations was observed only when eplerenone was added to incubation mixtures suggesting an increase or activation of digoxin metabolism in the presence of eplerenone. Depletion of digoxin was 24.5% and 27.8% with the low and high initial starting concentrations of 1.50 ng/mL and 7.50 ng/mL digoxin, respectively. The potential for clinical interaction resulting from eplerenone inhibition of digoxin metabolism is low.



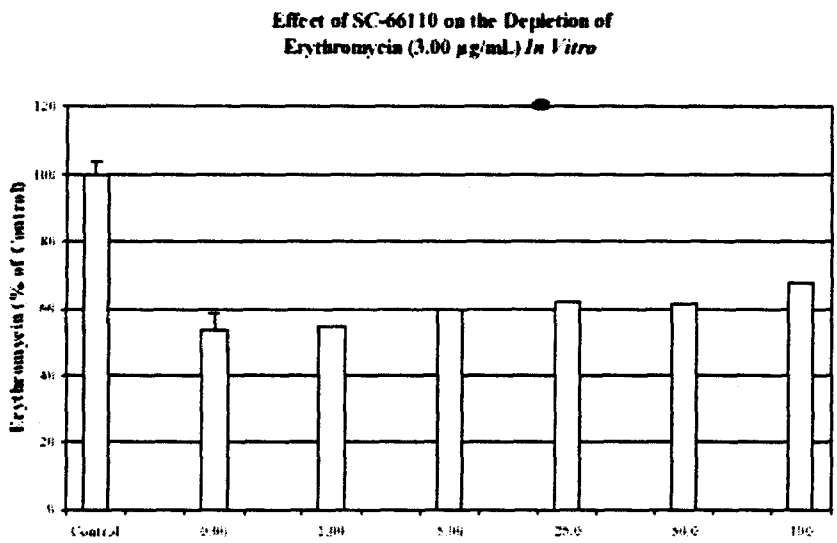


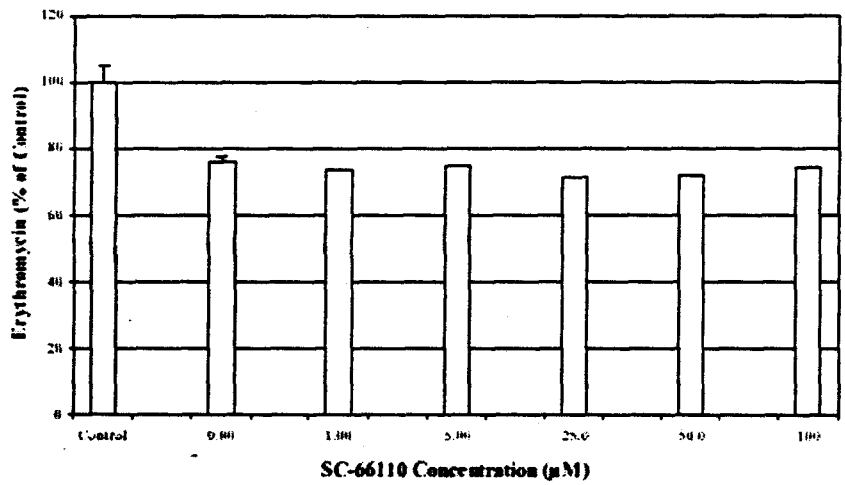
Digoxin did not inhibit the formation of SC-71597 when incubated at concentrations up to 75.0 µM therefore no Ki could be estimated. Since the therapeutic concentration for digoxin generally does not exceed 2.00 ng/mL, the potential for clinical interaction due to metabolic inhibition of eplerenone biotransformation by digoxin is low.

Potential for Interaction with Erythromycin

There was a concentration dependent trend toward an increased % remaining when eplerenone was added to incubation mixtures with the lower initial concentration of erythromycin although the concentration of eplerenone necessary to produce significant

inhibition was substantially higher than anticipated therapeutic plasma concentrations. This trend was not observed at the higher erythromycin concentration. The data indicate that alterations of erythromycin hepatic clearance due to coadministration of eplerenone are unlikely. In control incubations, about 46.7% or 24.2% of erythromycin (incubated at 3.00 or 15.0 mg/mL, respectively) disappeared during the 30 minute incubation in microsomes indicating substantial P450 dependent metabolism.

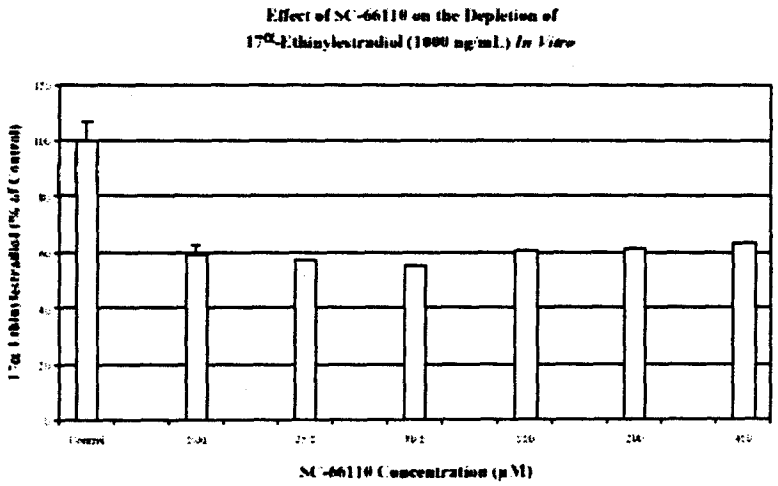




Erythromycin inhibited the formation of SC-71597 competitively with an estimated Ki of 9.47 µM, a level close to the plasma levels which have been reported clinically to approach 3 µg/mL (~4.1 µM) following chronic dosing. Therefore, a significant potential exists for decreases in SC-71597 formation and eplerenone clearance in the presence of erythromycin.

Potential for Interaction with 17a-Ethinylestradiol

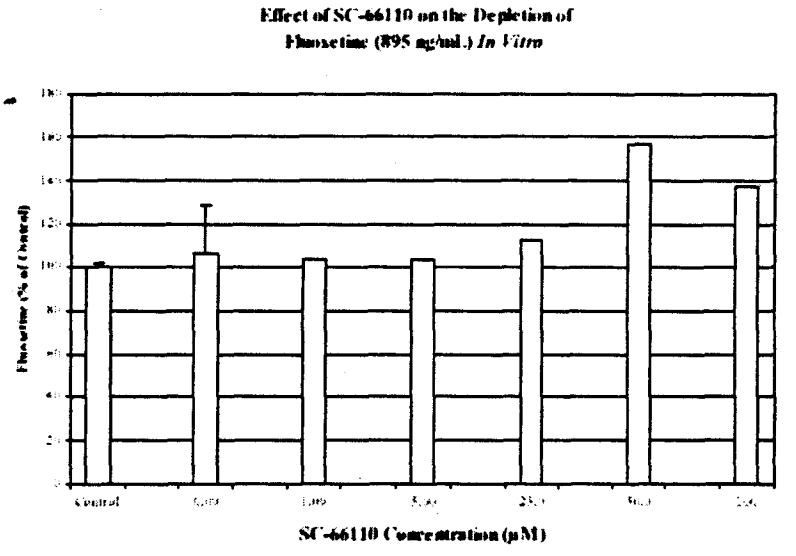
In control incubations, about 40% depletion of 17α-ethinylestradiol was observed with 58.3% and 59.5% remaining in incubations with low and high starting concentrations of 17α-ethinylestradiol, respectively. A small but concentration dependent trend towards higher amounts of 17α-ethinylestradiol remaining was observed with increasing concentrations of eplerenone. The potential for inhibition of 17α-ethinylestradiol metabolism by eplerenone appears unlikely since eplerenone concentrations used in the in vitro study exceeds anticipated plasma concentrations after therapeutic doses of 100 mg QD eplerenone.



The Ki estimated for inhibition of SC-71597 formation by 17α-ethinylestradiol was 19.5 μM estimated from a noncompetitive model. The estimated Ki value exceeds anticipated plasma concentrations at therapeutic doses, the Cmax reported following chronic doses of 35 mg/day is 125 pg/mL which is equivalent to 0.42 nM. A decrease in eplerenone clearance due to coadministration of 17α-ethinylestradiol appears unlikely.

Potential for Interaction with Fluoxetine

Eplerenone did not affect the metabolism of fluoxetine. In control incubations, complete recovery of fluoxetine was observed at the end of the incubation time consistent with its long half-life exceeding 48 hours. Additional experiments attempted to increase the metabolic depletion of fluoxetine by increasing enzyme concentration and incubation time were unsuccessful.

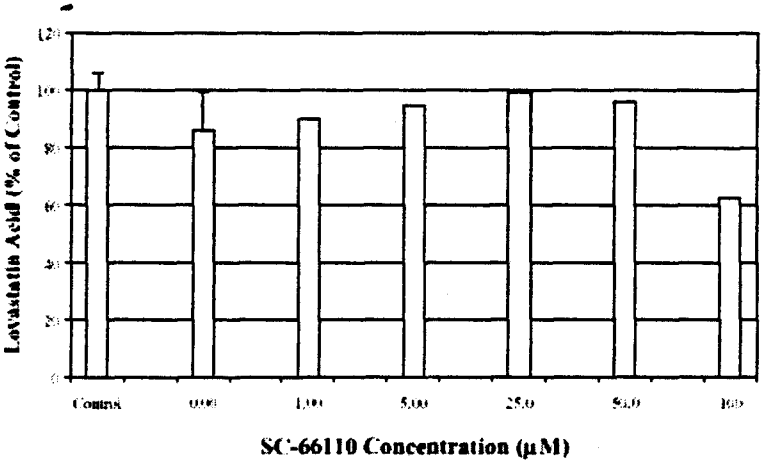


Inhibition of SC-71597 formation by fluoxetine was characterized by a linear-mixed model of inhibition with the associated Ki estimate of 17.6 μM. Anticipated maximal plasma concentrations of fluoxetine are approximately 200 ng/mL which is equivalent to ~580 nM. The enantiomers of fluoxetine and their circulating metabolites have been reported to inhibit CYP2D6, CYP2C19 and CYP3A4 with approximate inhibition constants of 0.60 μM, 0.20 μM and 83.3 μM respectively. Thus it appears that an interaction between fluoxetine and eplerenone due to metabolic inhibition is unlikely.

Potential for Interaction with Lovastatin/Lovastatin Acid

Lovastatin itself is metabolized to the pharmacologically active form lovastatin acid therefore lovastatin acid was monitored in studies of substrate depletion. In control incubations, less than 20% of lovastatin acid was depleted at the end of the incubation time. Eplerenone concentrations up to 25 μM decreased the disappearance of lovastatin acid resulting in near complete recovery of lovastatin acid. However, at highest concentration of eplerenone, 100 μM, nearly 40% of lovastatin acid was depleted at the end of the incubation time. These results suggest the presence of therapeutic concentrations of eplerenone is unlikely to affect the metabolism of lovastatin acid.

Effect of SC-66110 on the Depletion of Lovastatin Acid (50.0 ng/mL) *In Vitro*

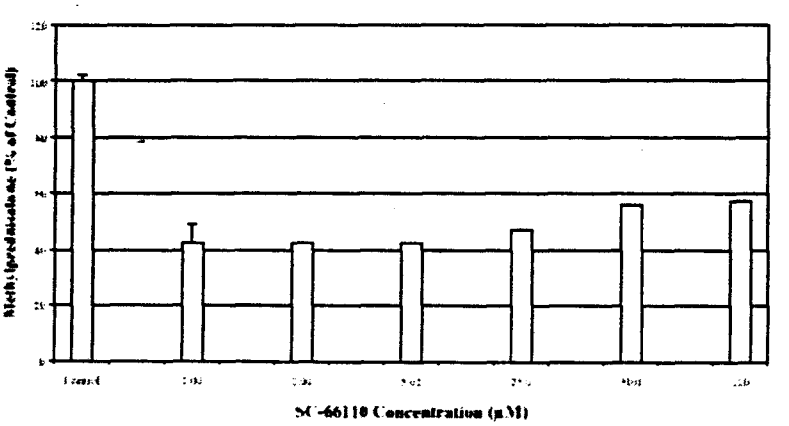


Inhibition of SC-71597 formation by lovastatin was described by a competitive model of inhibition with a Ki of 11.9 µM. Clinical inhibition of eplerenone clearance by lovastatin is unlikely since plasma concentrations of lovastatin are very low, <5 ng/mL after a 40 mg dose, compared to the estimated Ki.

Potential for Interaction with Methylprednisolone

Inhibition of methylprednisolone clearance was apparent as the percent remaining increased in a concentration dependent manner to 77.9% and 57.7% remaining, respectively, when 100 µM eplerenone was added to incubation mixtures containing high and low concentrations of methylprednisolone. In control incubations, the concentrations of methylprednisolone remaining in microsomal suspensions after 30 minutes incubation decreased by 37.1% or 42.5% of the respective low or high starting concentrations indicating substantial P450 metabolism. Although methylprednisolone metabolism was inhibited by eplerenone, the potential for an in vivo metabolic interaction is unlikely since eplerenone concentrations obtained following therapeutic doses (100 mg QD) are <

Effect of SC-66110 on the Depletion of Methylprednisolone (1000 ng/mL) *In Vitro*

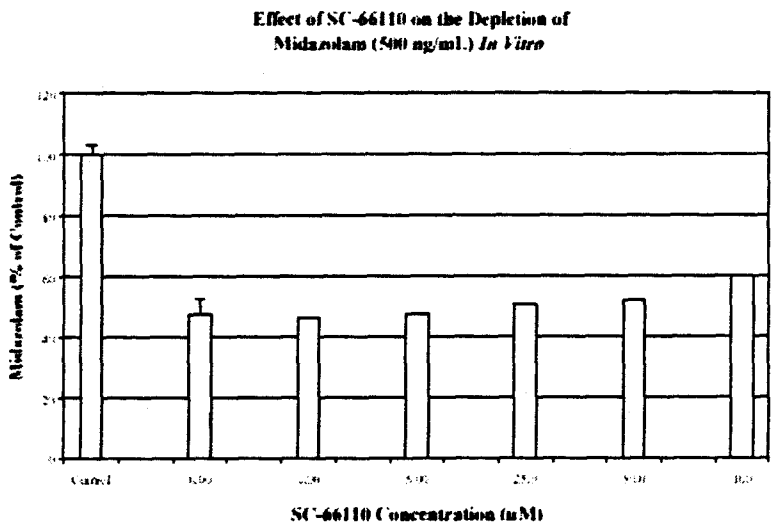


5 μM.

Methylprednisolone inhibited SC-71597 formation in a noncompetitive manner with an estimated Ki of 124 μM. An interaction with eplerenone via this mechanism is unlikely since plasma concentrations of methylprednisolone are low compared to the Ki, typically less than 200 ng/mL, equivalent to ~535 nM.

Potential for Interaction with Midazolam

A concentration dependent decrease in midazolam metabolism was observed in the presence of eplerenone. At the end of incubation with 100 ng/ml and 500 ng/ml of midazolam, 46.6% and 59.8%, respectively, of the initial concentration of midazolam were remaining in the presence of 100 μM eplerenone. In control incubations, when incubated at 100 ng/mL, 34.9% of midazolam remained after 10 minutes incubation while 47.6% remained in parallel incubations with starting concentrations of 500 ng/mL. The concentrations of eplerenone that resulted in measurable inhibition were significantly higher than its anticipated plasma concentration at therapeutic doses.

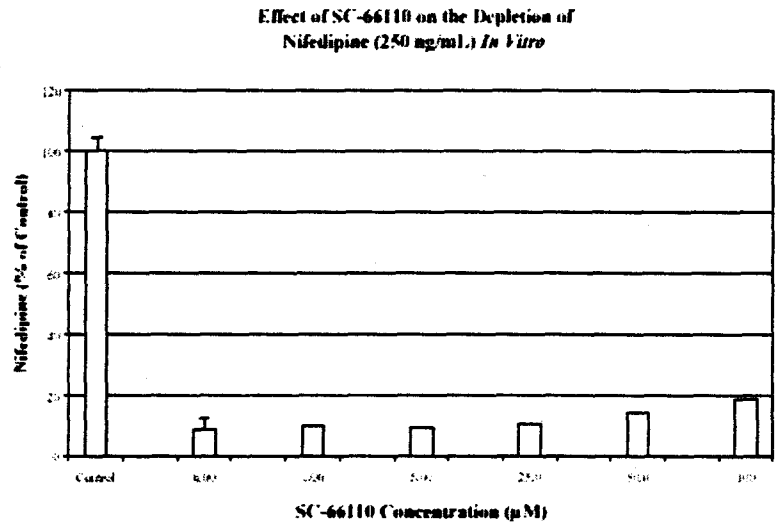


Midazolam inhibited the formation of SC-71597 in a noncompetitive manner with an estimated Ki for inhibition of 8.10 μM, a concentration in agreement with Km estimations reported for midazolam metabolism by CYP3A4 in human liver microsomes. Since typical plasma concentrations of midazolam are lower than the estimated Ki and do not exceed 200 ng/mL, equivalent to ~575 nM, the potential for significant clinical interaction due to inhibition by midazolam is expected to be minimal.

Potential for Interaction with Nifedipine

In control incubations, nifedipine was rapidly and extensively depleted from microsomal suspensions with only 9.98% and 8.36% remaining with respective starting concentrations of 50.0 or 250 ng/mL, respectively. Concentration dependent reduction of

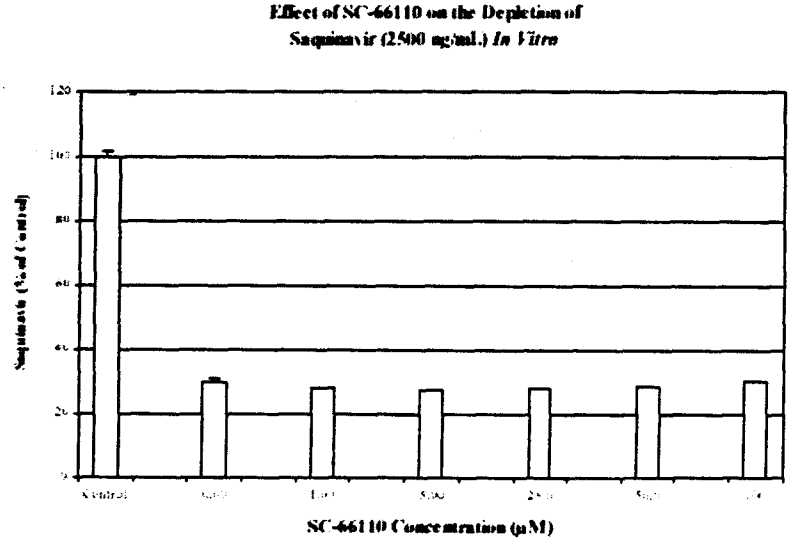
nifedipine disappearance was observed when eplerenone was added to incubation mixtures regardless of initial nifedipine concentration with 18.8% and 18.5% remaining in incubations with respective initial concentrations when eplerenone 100 µM was included. Nifedipine concentrations are not likely to be affected in vivo upon coadministration with eplerenone since therapeutic doses of eplerenone yield concentrations <5 µM.



Nifedipine inhibited SC-71597 formation non-competitively with an estimated Ki of 21.8 µM. This concentration substantially exceeds typical nifedipine plasma concentrations; nifedipine Cmax was 124 ± 51 ng/mL equivalent to ~360 nM following a single oral dose of 10 mg which is lower than the estimated Ki of 21.8 µM. Thus, inhibition of SC-71597 formation in vivo by nifedipine is expected to be unlikely.

Potential for Interaction with Saquinavir

Except for the slight inhibition in saquinavir disappearance, 17.7% remaining, at the highest concentration of eplerenone (100 µM), eplerenone did not affect the disappearance of saquinavir at lower concentrations. In control incubations, 9.94% and 29.7% of saquinavir remained with starting concentrations of 500 ng/mL and 2500

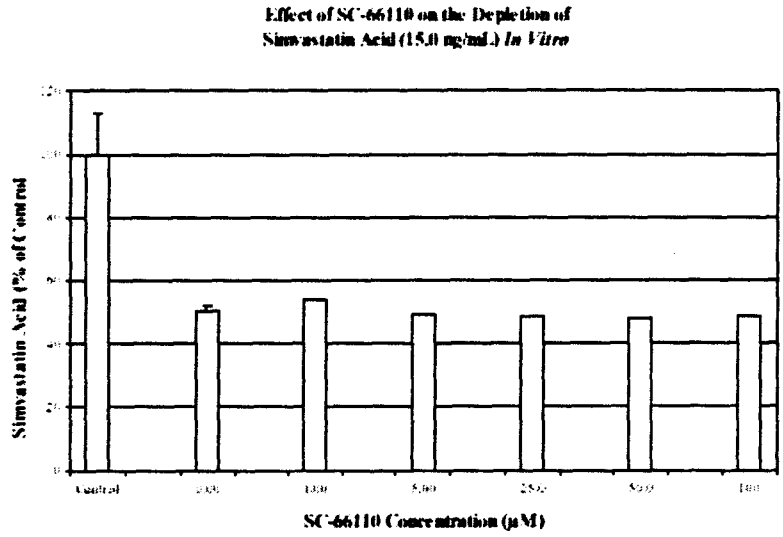


ng/mL, respectively. A change in the clearance of saquinavir due to inhibition by eplerenone is unlikely at therapeutic concentrations of eplerenone.

Saquinavir proved to be a potent linear-mixed inhibitor of SC-71597 formation with a Ki estimate of 546 nM. This Ki is within the range of therapeutic concentrations expected with chronic treatment of 600 mg TID which yields concentration of 100 -500 ng/mL equivalent to ~130-650 nM. Thus, it is likely that eplerenone clearance will be decreased in the presence of saquinavir.

Potential for Interaction with Simvastatin/Simvastatin Acid

There were no differences in simvastatin acid disappearance in the presence of eplerenone suggesting that changes in simvastatin acid metabolism are unlikely due to coadministration of eplerenone. In control incubations with initial simvastatin acid concentrations of 3 ng/mL and 15 ng/mL nearly 50% of simvastatin acid was metabolized within 30 minutes in microsomal suspensions.

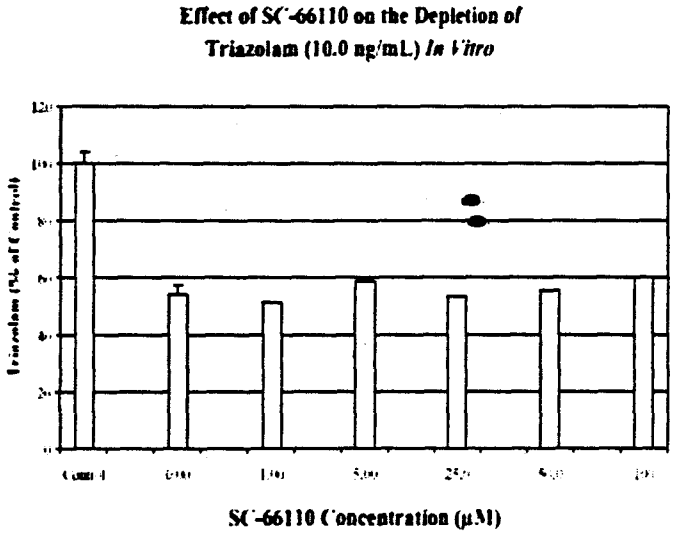


Simvastatin competitively inhibited SC-71597 formation from eplerenone observed when simvastatin was added to incubation suspensions. Using a competitive model of inhibition a Ki of 6.23 μM was estimated. The estimated Ki exceeds the expected therapeutic concentration of simvastatin of 10-30 ng/mL equivalent to ~25-75 nM. Therefore, clinical interactions resulting from simvastatin inhibition of eplerenone metabolism are expected to be unlikely.

Potential for Interaction with Triazolam

Eplerenone concentrations up to 100 μM did not affect the disappearance of triazolam. Incubates containing low and high triazolam concentrations 52.7% and 60.0% of initial

triazolam concentrations were remaining in the presence of eplerenone 100 μM. In control incubations, 50.7% and 54.0% of triazolam concentrations remained in incubates with low or high starting concentrations of triazolam, respectively, indicating significant P450 dependent metabolism of triazolam. No clinical interactions are anticipated between eplerenone and triazolam in vivo.



Triazolam competitively inhibited SC-71597 formation with an estimated Ki of 408 μM. Triazolam plasma concentrations are expected to be in the nanomolar range with Cmax reaching 7.81 ± 2.59 ng/mL equivalent to ~23 nM following daily doses of 0.5 mg. These concentrations are lower than the estimated Ki of 408 μM. Therefore metabolic inhibition of eplerenone by triazolam interaction is not expected in vivo.

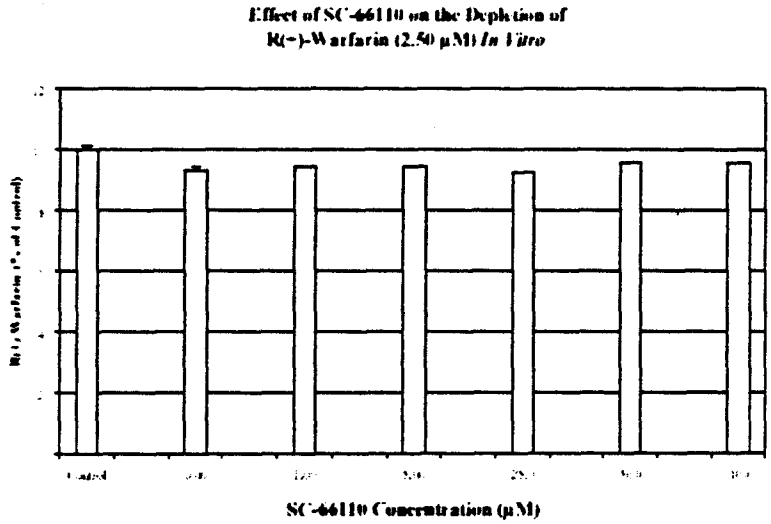
Potential for Interaction with Verapamil

Addition of eplerenone resulted in concentration dependent inhibition of verapamil disappearance regardless of initial verapamil concentration. At the end of incubation with 100 μM eplerenone 9.25% and 16.9% of the low and high initial concentration of verapamil remained. While, in control incubations, nearly all the verapamil was metabolized in 30 minutes with only 1.70% and 6.67% remaining in incubates with starting verapamil concentrations of 200 ng/mL or 1000 ng/mL, respectively. At therapeutic concentration of eplerenone (<5 μM) eplerenone is not expected to affect the metabolism of verapamil in vivo.

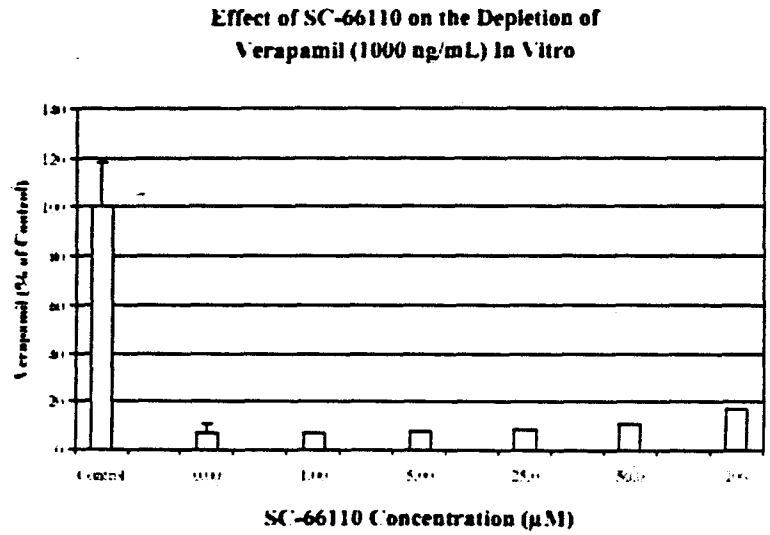
Verapamil inhibited the SC-71597 formation competitively with an estimated Ki of 13.3 μM. Based on expected therapeutic concentrations of verapamil of approximately 500 nM which is lower than the estimated Ki of 13.3 μM, formation of SC-71597 is not expected to be affected by verapamil, however, drug interactions with verapamil are complex and may involve circulating verapamil metabolites or alterations in activity of transporter proteins.

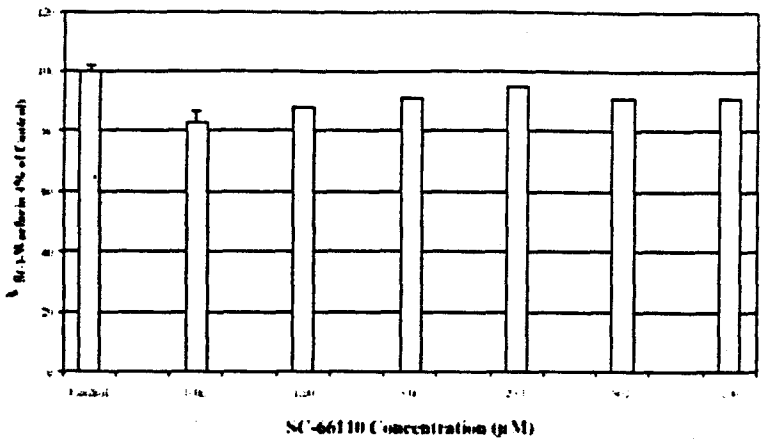
Potential for Interaction with (R)-Warfarin

In control incubations, (R)-warfarin metabolism was negligible consistent with its long elimination half life of 48 hours. Eplerenone concentrations up to 100 μM did not affect (R)-warfarin disappearance. Clinical interaction of eplerenone on the metabolism of (R)-warfarin is not expected.



(R)-warfarin competitively inhibited the formation of SC-71597 only at high concentrations with an estimated Ki was 784 mM. The estimated Ki value exceeds the therapeutic concentrations of (R)-warfarin. Therefore no change in SC-71597 formation





is likely to occur as a result of warfarin coadministration.

Potential for Interaction with (S)-Warfarin

Eplerenone concentrations of 100 µM decreased the metabolism of (S)-warfarin to 14.1% and 9.2% of initial low and high concentrations of warfarin. In control incubations about 20% of (S)-warfarin was metabolized during the incubation. Disappearance of (S)-warfarin was 23.0% and 17.4% in incubations with low or high initial levels of (S)-warfarin, respectively. Based on the low therapeutic concentrations of eplerenone (<5 µm), a significant effect on (S)-warfarin metabolism is not expected.

(S)-warfarin inhibited the formation of SC-71597 competitively with a Ki of 750 µM. This concentration greatly exceeds the therapeutic plasma concentration of (S)-warfarin. Consequently, no changes in SC-71597 formation are anticipated due to coadministration of warfarin.

CONCLUSIONS:

Based on the results of the present in vitro drug interaction study with 19 different substrates, eplerenone at therapeutic concentrations is expected to affect the metabolism of digoxin only. Also, cyclosporin, erythromycin and saquinavir are expected to affect the formation of SC-71597 from eplerenone in vivo.

Clinically relevant drug interaction between eplerenone and amlodipine, astemizole, cisapride, diazepam, 17α-ethinylestradiol, fluoxetine, lovastatin, methylprednisolone, midazolam, nifedipine, simvastatin, triazolam, verapamil and warfarin are not expected in vivo.